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DETECTION OF TURBIDITY IN AMNIOTIC FLUID BY AN ULTRASONIC TECHNIQUE

BY

PAAVO PYSTYNEN, KAI AF URSIN AND ANTTI SAVOLAINEN

Introduction

The frequency range of audible sound is 30–15,000 cycles per second, whereas the frequencies of ultrasound are much higher and those generally used are between 200,000 and 15,000,000 cycles per second. These frequencies cannot be detected by the human ear. Compared with audible sound the frequencies of ultrasound are not only higher but the wave lengths are shorter. Due to these properties the penetrating and echoing abilities in various media are better, depending to a certain extent on the frequency and the intensity used, i.e. the amplitude of the wave. Since World War II ultrasound has been extensively used in warfare, traffic, fishing, and industry etc. During the past 15 to 20 years it has also been employed in medicine, especially in differential diagnosis. From a survey of the English literature it would appear that gynecologists and obstetricians have shown considerable interest in this aspect of ultrasound. Ultrasound has been used during the last ten years to diagnose intracranial lesions and also in the diagnosis of eye diseases. In ophthalmology the use of ultrasound has become a routine procedure, and the Ophthalmological Department of the Central Hospital of Tampere, Finland, has had an ultrasound machine since September 1963. This machine was used in the present obstetrical investigation.

The turbidity of the amniotic fluid has not been examined by ultrasonic methods before. The ultrasound method (in one

dimension) used in this study is especially suitable for the study of fluids in this case that of the amniotic fluid. The apparatus also permits vaginal examination, the results of which appear to be important. The ultrasound equipment with the scanning principle intended for diagnosis of tumours and foetal status would not for reasons of size and shape be suitable for vaginal examination

Method and aims

The equipment used was constructed by an Austrian engineer Kretz, for ophthalmological work (Fig 1) It differs from the machine based on the scanning principle in that it is not possible to "photograph" the studied object directly A small mobile barium titanate crystal a so-called sound modifier is pressed against the cornea, or against the skin, or mucous membrane as in the present work The crystal modifies the electrical waves produced by the equipment to ultrasonic impulses of high frequencies When the pulsed ultrasound wave meets the interface *of two materials with different densities part of the ultrasonic* impulse is returned to the crystal, which now acts as a receiver These echoes are transformed to electrical signals which can be projected on to a cathode ray oscillograph. Here the signals are recorded as vertical waves and can be photographed. They may also be recorded on cine-film. In the present work a cine-film method developed by af Ursin (1964) was used. The film recorded 12 exposures a second and one exposure of every film taken is shown in this paper This sort of registration of the results naturally is only of research value In practice the results are read on the oscillograph during the examination.

Since turbid states of the vitreous humor can be detected with this ultrasound method (Oksala and Lehtinen, 1958 1959 Oksala 1959 Löpping, 1963 Stallkamp 1961 and af Ursin, 1964) the question was raised as to whether this method could be applied to detect turbid states of amniotic fluid. When amniotic fluid is meconium stained, for example *in acute asphyxial states of the foetus*, the fluid may be turbid and contain aggregations In postmature pregnancies vernix caseosa may sometimes be present in the amniotic fluid.

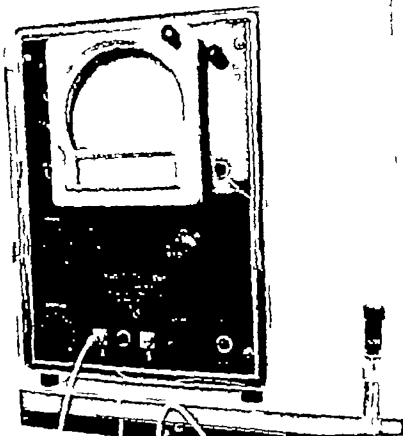


Fig. The equipment employed in the ultrasonic investigations. Right, the crystal, 6 mHz and .2 cm in diameter

A suitable crystal had first to be chosen, because naturally the crystals used in ophthalmology were not applicable to an examination made through the abdominal wall or the vagina, as the ultrasound here had to penetrate relatively thick layers. Prof Oksala, 1964 an expert in ophthalmological ultrasound techniques suggested that the crystal should be 6 mHz and 1 2 cm, or 2



Fig. 2. Oscillograph of distilled water The echoes on the left are due to the surface of the water those on the right to the bottom of the glass container The area between these two deflections, with no echoes, represents the distilled water

mHz and 2.4 cm in diameter Both sizes were obtained and the smaller one appeared to be the more suitable.

Material and results

Amniotic fluid was tested in three ways (a) *in vitro* (27 samples) (b) *in vivo* through the abdominal wall (56 patients) and (c) *in vivo* through the vagina (44 patients) thus the series consisted of 100 examinations.

(a) *Amniotic fluid in vitro (27 samples)*

This test was required to determine the penetrating ability and behaviour of the ultrasound wave when the new crystal was used. Also a comparison of these results and those obtained from *in vivo* examination, was deemed necessary The crystal was immersed in distilled water (Fig 2) and in clear amniotic fluid

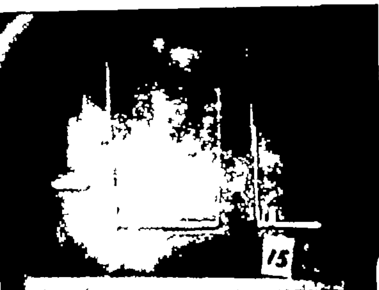


Fig. 3. Clear amniotic fluid obtained by paracentesis. The interpretation is similar to Fig. 2.

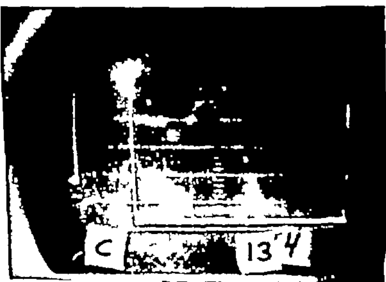


Fig. 4. Oscillograph of two superimposed placental layers. N echoes are visible.



Fig. 2 Oscillograph of distilled water The echoes on the left are due to the surface of the water those on the right to the bottom of the glass container The area between these two deflections, with no echoes, represents the distilled water

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Fig. 6 Examination in vivo through abdominal wall. Clear amniotic fluid was obtained by paracentesis immediately following the ultrasound examination. The broad echoes represent the solid tissues between the skin and the amniotic fluid. The fluid gave no echoes even with an amplitude of o. The foetal component of the oscillatory pattern cannot be seen in this view. It was visible when the field was moved to the right to a point where the abdominal wall component disappeared. This was case of polyhydramnios.

contact gel was used to apply the crystal to the site where manual examination had revealed the greatest amount of fluid. The interposing layer between the skin and the amniotic fluid comprises the abdominal and the uterine wall. The crystal was pressed against the skin with varying force the amniotic fluid thus dispersed between the membranes and some part of the foetus. The echo produced by the foetus could be differentiated from other echoes in this way

Of the 56 patients examined, 46 showed no echoes on the screen, and the fluid was thus judged to be clear This was confirmed in 38 cases by visual examination of the amniotic fluid during delivery In 2 patients the clarity of amniotic fluid was confirmed by paracentesis, and in another 2 patients artificial rupture of the membranes, immediately following the ultrasound



Fig. 5. Oscillograph of amniotic fluid to which meconium, in the ratio of 1 gm to 40 ml of amniotic fluid, has been added. This test was also carried out through two superimposed placental layers as in Fig. 4. The amplitude was 5. Clear and high echoes are distinctly visible.

(Fig 3) obtained by paracentesis No echoes were noted in the oscillograph. The same test was performed through one, two and three superimposed placental layers (Fig. 4) and also through uterine muscle 1 cm thick and then through both uterine muscle and placenta together The results were the same When increasing concentrations of meconium were added to the sample fluid, oscillations were clearly seen on the screen (Fig. 5) The results were similar when the test was made through the layers listed, and even when the fluid was only slightly turbid.

(b) *Amniotic fluid in vivo tested through the abdominal wall (56 patients)*

As the preliminary tests appeared promising, a study of amniotic fluid in late pregnancy was begun. This study can be done in two ways either by applying the crystal to the abdominal wall or inserting it into the vagina. In the former method Methocell

Fig. 8. Examination *in vivo* through abdominal wall. Foetal heart sounds were varying and meconium was anticipated in the amniotic fluid. Many echoes are projected to half the height of those representing the abdominal wall and the foetus. The membranes were ruptured artificially after the examination and the amniotic fluid was seen to be meconium-stained.

Fig. 9. Examination *in vivo* through abdominal wall, performed after spontaneous rupture of the membranes. The fluid ...



Fig. 7 Examination *in vivo* through abdominal wall. The clarity of the amniotic fluid was verified on artificial rupture of the membranes immediately after the examination. The broader echoes represent the abdominal wall, the narrower the foetal components. Between these two areas no echoes are visible. With increased amplitude the picture remained unchanged. With pressure variation of the crystal the foetal component moved on the screen, while the abdominal wall component remained stationary

test showed the amniotic fluid to be clear The ultrasound recording of one patient treated by paracentesis is shown in Fig. 6 and that of one with artificially ruptured membranes in Fig 7 no echoes were obtained. In 4 patients absolute confirmation of the result of the ultrasound test was unobtainable because of the paucity of amniotic fluid.

In the remaining 10 patients of the total of 56 in this group green amniotic fluid was seen during delivery In one case the ultrasound recording was performed immediately before artificial rupture of the membranes and several clear echoes were seen (Fig 8) The finding was confirmed as soon as the membranes were ruptured and the green-coloured amniotic fluid became evident. The membranes of 9 patients had ruptured spontaneously before the ultrasound test, but there was sufficient residual

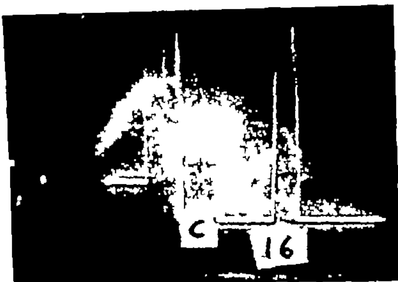


Fig. 12. Vaginal examination, revealing meconium in the amniotic fluid. Amplitude The echoes produced by the meconium are not visible at this low intensity

amniotic fluid for definite echoes to be recorded with the ultrasound technique in 8 cases (Fig. 9) In the remaining case there was insufficient fluid for an ultrasound recording.

(c) *Amniotic fluid in vivo through the vagina (44 patients)*

The crystal was placed in the tip of a rubber finger stall containing one drop of Methocell gel. The finger stall with the crystal was then introduced into the anterior fornix of the vagina. Previously it had been demonstrated that the results were the same with and without the glove, i.e the glove did not alter the results. This examination is most suitable in cases where the fœtal head has not engaged. The crystal was kept still, while the assistant lifted the fœtal head longitudinally thus keeping a certain amount of amniotic fluid between the fœtal head and the membranes. The echo seen on the screen which was produced by the head moved with head movements.

The ultrasound method revealed clear amniotic fluid in 41 cases, confirmed during delivery In 2 cases low voltage echoes

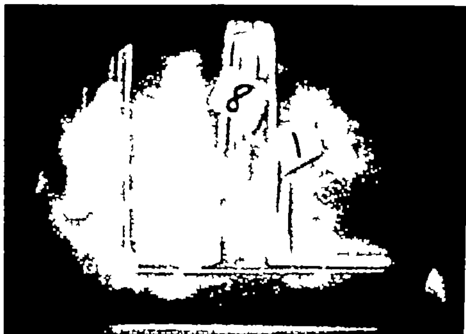


Fig. 10. Vaginal examination, showing clear amniotic fluid. The echoes on the left are due to the uterine isthmus. The broad echoes represent the fœtal head. These echoes moved to the right when the head was lifted sagittally and to the left when the head was allowed to engage. The fluid remaining between the uterine muscle and the fœtal head gave no echoes even with the greatest amplitude.

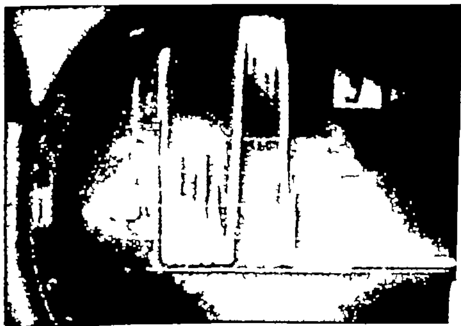


Fig. 11 Vaginal examination. Amplitude 2 1/2 gave no echoes whereas amplitude 5 gave low but definite oscillations. On Caesarean section the fluid was seen to contain aggregations of vernix caseosa.

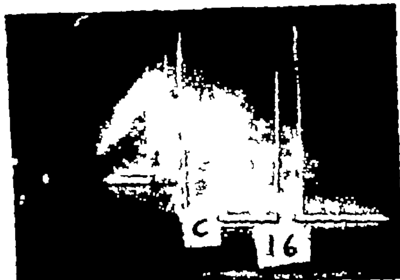


Fig. 12. Vaginal examination, revealing meconium in the amniotic fluid. Amplitude 1. The echoes produced by the meconium are not visible at this low intensity

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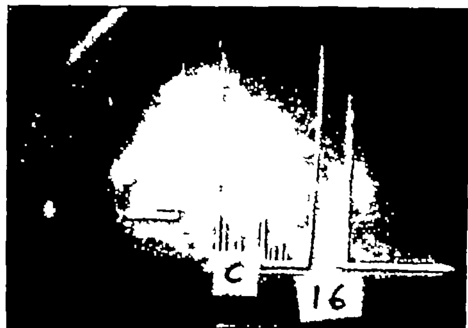


Fig 13 The same case as in Fig. 12. When the amplitude is increased the echoes begin to show The amplitude used here is 2.

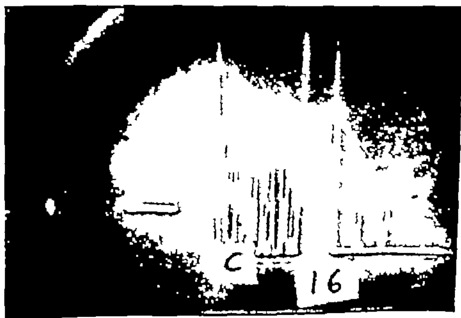


Fig 14. The same case as in Figs. 12 and 13. Amplitude 3. The echoes are higher than in Figs. 12 and 13.



Fig. 5. The same case as in Figs. 2, 13, 14. Amplitude 5. The echoes ar even higher

were seen on the oscillograph. In one of these cases the fluid was stained with vernix caseosa and this confirmed at subsequent Caesarean section. In the other case the turbidity of the fluid was not confirmed at delivery. Fig. 10 shows echoes of a case with clear amniotic fluid. Fig. 11 illustrates the case with fluid stained by vernix caseosa and delivered by Caesarean section. In the remaining case, a postmature one, the foetal heart sounds showed sudden large variations. Labour had just started and the head had not engaged. Paracentesis was performed immediately and the amniotic fluid was green, meconium-stained. A vaginal *ultrasound* examination was performed immediately increasing the amplitude gradually from 0 to 5. The results are seen in Figs. 12–15. The membranes were artificially ruptured, and fluid was seen to be green. The child was stillborn.

Discussion

The results of ultrasound examination are read routinely on the oscillograph, although they were photographed for presenta-

tion in this study In using the method experience is necessary

The *in vitro* results are conclusive clear amniotic fluid produced no echoes whereas meconium-stained fluid gave definite echoes, even when in low concentrations and tested through thick layers.

Most probably meconium is evenly diffused through the whole sac of amniotic fluid, especially in asphyxial states with excessive foetal movement. There are numerous small particles in the fluid which get in the way of ultrasound they can be seen as narrow peaks on the oscillograph and fill the examined section of amniotic fluid completely from one end to the other The echoes produced by these particles are not strong if recorded through the abdominal wall

In vivo examinations through the abdominal wall gave no echoes with clear amniotic fluid but did with green fluid. The cases with inconclusive results may be attributable to the small amount of amniotic fluid, which made it impossible to differentiate between the influences of the abdominal wall the amniotic fluid and the foetus on the oscillatory pattern. The thickness of the amniotic fluid layer between the membranes and the foetal part is decisive the nature of the foetal part is not. The echo produced by the head, trunk, extremity or umbilical cord is strong Ultrasound produces echoes of different height when waves are directed from different angles to the same object. By changing the position of the crystal it is possible to find the highest possible echo This echo low or high moves with the rhythm of the pressure produced by pressing the crystal against the abdominal wall with variable force The part between the skin and the membranes remains immobile. This phenomenon is explicable because the thickness of the amniotic fluid layer between the foetus and the membranes varies accordingly If amniotic fluid gives no echoes it may be said to be clear Ex amination through the abdominal wall is not so well suited in routine work to positive detection of meconium in the amniotic fluid, but it does exclude the possibility of turbidity caused by confluent meconium particles.

It seems that vaginal examination provides information about the turbidity of the amniotic fluid only as long as the head has

not engaged. Examination made through the abdominal wall and in vitro has facilitated an understanding of the results obtained by vaginal examination, which may acquire practical importance in the future. The method is not needed in the delivery room but for patients admitted for some special reason before labour the clarity of the amniotic fluid is of interest. Since the foetal head is often not engaged in these cases, the examination can be performed advantageously. The results obtained seemed reliable, and the crystal 6 mHz and 1.2 cm in diameter appeared optimal. This method is simple and sterile. The patient need not be moved to a gynaecological table. Rupture of the membranes and/or induction of labour need not be feared since the uterine endocervix is not touched: this method is therefore preferable to amnioscopy. Provided the presenting part of the foetus has not engaged and can be lifted, the vaginal method is more reliable than the abdominal. The fact that the amniotic fluid is separated from the crystal by only a thin layer of uterine muscle (the isthmus) allows detection of even slight turbidity.

It might be better to use first the lowest intensity of ultrasound and increase it rapidly to the maximum available (amplitude 10). Higher amplitudes were necessary (5–10) for examination through the abdominal wall while lower intensities sufficed in vaginal examinations. In the case with vernix caseosa in the amniotic fluid, no echoes were recorded with amplitudes under 5: in this case aggregations in the fluid were very small. But in the case with meconium-stained amniotic fluid the echoes were recorded with an amplitude of 1 and over. The intensity of the ultrasound, the height, breadth and frequency of the oscillations can give qualitative indications of the turbidity present.

The equipment required weighs only 25 kilograms and is readily transportable. The examination is painless, quick and safe to both mother and child if only the frequencies mentioned are used: this was recently demonstrated by Sundén (1964) in animal experiments. This technique is mainly used in cases where labour has not yet commenced and amniotic fluid has not drained. The limitations of the method should be appreciated: the bilirubin content of the amniotic fluid cannot be detected,

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diagnosis of asphyxial foetal states and as experience is accumulated it may be of value in the diagnosis of postmature pregnancies.

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nor can green discoloration of the fluid unless there are aggregations which always occur in severe asphyxial fœtal states. According to *in vitro* tests, meconium remains undiluted or undispersed in the amniotic fluid for several hours.

It must be appreciated that the present study was carried out with equipment originally designed for ophthalmological work, although it appears to be well suited for amniotic fluid studies. The dimensions and physical properties of the crystal used seem to be suitable for examining amniotic fluid through the vagina. On the other hand, examination through the abdominal wall would require better penetrating ability and less absorption of the ultrasound by the abdominal wall.

SUMMARY

The clarity and turbidity of amniotic fluid was studied by the ultrasound method (one-dimensional) *in vitro* (27 tests) and *in vivo* both through the abdominal wall (56 patients) and through the vagina (44 patients) The equipment used was designed by Kretz for ophthalmology The crystal employed was 6 mHz and 1 2 cm in diameter and it seemed well suited for vaginal examination for abdominal examinations its properties needed to be modified.

The study showed the following

1 *In vitro*, even minimal amounts of meconium in the amniotic fluid could be detected.

2. *In vivo* examination through the abdominal wall is not so well suited in routine work to positive detection of meconium in the amniotic fluid, but it does indicate the clarity of the fluid by excluding the possibility of turbidity

3 Vaginal examination seems to be the most suitable method in practice. The clarity or turbidity of the amniotic fluid can be shown, the method is easy reliable quick and safe. As long as the presenting part has not engaged, it can be lifted leaving a definite layer of amniotic fluid between the lowest pole of the foetal sac and the presenting part of the fœtus. With this technique the ultrasound method can be used for detecting meconium-stained amniotic fluid. It appeared to be of value in the

picion of placenta praevia, on clinical grounds, and admitted to this department, was examined by means of arteriography The series was analysed to obtain a clear idea of the diagnostic value of the method and to study the prognosis for mother and child both in cases of placenta praevia and in cases where the source of bleeding was not determinable by existing methods.

Radiologic Technique

Premedication. All patients received a sedative prior to examination. A preparation of the phentiazine-group was found most suitable owing to its low toxicity and good sedative and antiallergic properties. Usually 50 mg. of Lergigan (Promethazin. chlorid., Recip) was administered orally on the evening before examination. In acute cases 25 mg. of the same preparation was injected intramuscularly about one hour before examination. If the patient was very sensitive and anxious 50–100 mg. of *Pethidine* intramuscularly was also given 10–15 minutes before beginning the arteriography It has been shown that uterine contractions may prevent or reduce the filling of the intervillous spaces with contrast medium (Borell, Fernström and Ohlson, 1963) In patients having uterine contractions we have consequently given 5–10 mg. of isoxysuprinchloride (*Duvadilan*, Ferrosan) intramuscularly about 10 minutes before the contrast injection in an attempt to reduce uterine activity

Introduction of the catheter The contrast medium was injected through a yellow or grey Ödman-catheter which is introduced by means of the instrument, devised by Seldinger after puncture of the femoral artery under local anaesthesia. The Ödman-catheter has side holes which results in a more uniform spread of the opaque medium in the aorta and facilitates a rapid injection. The catheter is passed up to the level of the aortic bifurcation, that is 20–25 cm. from the site of puncture. If the catheter is passed further up in the abdominal aorta too much of the contrast medium is lost to the renal arteries to get a good visualization of the placental sinuses With the catheter at the suggested level, the opaque medium usually will pass up just to the level of the renal arteries, thus allowing visualization

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HÆMORRHAGE IN LATE PREGNANCY

With special reference to angiographic localization of the placenta

BY

ERIK HEDBERG AND CLAES RÅDBERG

Antepartum hæmorrhage is still an important cause of maternal mortality Many of the deaths due to hæmorrhage can be prevented by proper antenatal supervision and institutional care. The decreased maternal mortality from hæmorrhage in recent years can be ascribed mainly to a higher standard of antenatal care the increased use of blood transfusion, effective chemotherapy and a better understanding of the management of shock. But an important contributory factor is the development of techniques for localizing the placental site and consequently the origin of the hæmorrhage In this respect an early and correct diagnosis of placenta prævia is of the utmost importance.

It has been generally accepted that antepartum hæmorrhage should be regarded as caused by placenta prævia until this condition has been excluded. Different methods are used for diagnosing placenta prævia and in recent years the use of radiologic methods as an aid to diagnosis has increased. Of the radiologic methods available arteriography has proved to be of the greatest value for the localization of the placenta (Harnett, 1948 Norman 1953 Fernström 1955) The reliability of the method has been discussed by a number of authors and detailed results of large series have been published (Brink 1948 Borell Fernström and Ohlson, 1963 Basson and de Villiers 1963)

Since 1960 each case of antepartum hæmorrhage with a sus-

visible. By means of stereoscopy and by studying the parallactic displacement the position of the placenta is determined.

Technical data. The mean exposure time was 0 10–0 12 seconds, using 500 Ma and 75–80 pKv With the exposure data mentioned above the maternal ovaries receive 0 2–0.3 r at each stereoscopic exposure-pair i e the total gonadal radiation dose for the whole examination is 0.4–0.6 r

Radiologic definition of different types of placenta praevia. The upper border of the lower uterine segment may be related to bony land marks and is posteriorly at the level of the promontory and anteriorly at a point 4–5 cm above the symphysis (Borell, Fernström and Ohlson, 1963) The site of the internal os was estimated from the films taken in the arterial phase and was considered to be at the level where the uterine arteries turn upwards along the lateral margin of the uterus.

If no placental sinuses are seen below the upper limit of the lower uterine segment, placenta praevia is not present. A low lying placenta is diagnosed if placental sinuses are visible within 3 4 cm. below the upper margin of the lower uterine segment. A total placenta praevia is diagnosed if the intervillous spaces extend to or below a plane parallel to the upper limit of the lower uterine segment and passing through the internal cervical os. Owing to the difficulties in distinguishing between the marginal and partial type of placenta praevia these two groups are put together under the heading of marginal placenta praevia. This group includes all cases with the placenta localized between low-lying and total placenta praevia as defined above

In those cases where the intervillous spaces are visible below the level of the promontory on the roentgen picture it is of course necessary to state whether the placenta is implanted on the posterior or anterior wall since the lower segment of the uterus extends higher posteriorly than anteriorly According to Borell *et al.* such a determination requires another injection of contrast medium and a lateral exposure of the pelvis. Owing to the density of the body in this projection this exposure involves a fairly high radiation effect and consequently greater

of the ovarian arteries, which usually originate either from the abdominal aorta some cm below the origin of the renal arteries or from the renal arteries.

Both femoral arteries are compressed during the injection of the contrast medium in order to improve the filling of the uterine arteries and to prevent loss of contrast medium into the vessels of the legs

Contrast medium As contrast medium a 60 per cent solution of *Urografin* (Schering) was used. 50 ml of the solution was injected within 2 seconds by means of a pressure syringe with a pressure of 4–5 kg

Exposure factors With an automatic film-exchanger two pairs of stereoscopic films (all together 4 films) were taken by means of a stereo-roentgen tube with an angle of 8 between the central beams The first pair of films was taken at an interval of one second, when about 30 ml. of the contrast medium had been injected The next pair of films was taken 3–4 seconds later with an interval of half a second.

The first pair of films illustrates the arterial phase. The comparatively long interval between the films does not usually permit stereoscopic viewing but on these films the hypertrophic arteries which supply the placenta are visible. It is also possible to deter mine the position of isthmus and the internal cervical os usually situated at the level where the uterine arteries reach the uterine wall and bend up along the lateral margin of the uterus.

In some cases the uterus and placenta are supplied mainly by the ovarian arteries and in those cases a satisfactory contrast filling of the ovarian arteries is of utmost importance to get a true idea of the site and extension of the placenta. To judge the contrast filling of the ovarian arteries the field of visualization must cover almost the entire uterus. If the ovarian arteries are not filled and it seems likely that they contribute to the placental blood supply another contrast injection has to be made with the catheter placed in a position more favourable for filling of the ovarian arteries

On the second pair of films opacified placental sinuses are

in 66 cases. In two cases the placental site could not be definitely determined radiologically. Both were full-term pregnancies where the uterine contractions caused unsatisfactory filling of the placental sinuses. The clinical course suggested a normal implantation of placenta in both cases.

The cases of placenta praevia or low-lying placenta were classified as

Low-lying placenta	26 cases
Marginal placenta praevia	30 cases
Total placenta praevia	10 cases

Attempts have been made in earlier publications to correlate the radiologic diagnosis with clinical findings at vaginal palpation, Caesarean section, inspection of the expelled placenta and site of rupture of the membranes. We have found it most difficult to perform such a correlation as most of the clinical methods for the diagnosis of placenta praevia include various kinds of errors. Only in connection with Caesarean section can the placental position be determined. In the present series Caesarean section was performed in 34 cases. In 30 of these cases the hospital records gave a detailed description of the placental site and in all cases the radiologic diagnosis was confirmed. In those cases where arteriography had shown a normal implantation of placenta the clinical course confirmed the radiologic diagnosis.

Treatment

All patients with diagnosed placenta praevia or low-lying placenta were treated expectantly until the foetus was considered viable or until severe haemorrhages forced interruption of the pregnancy. All the patients were kept in bed at the hospital until the bleeding had ceased. If no further vaginal bleeding occurred within a week they were allowed to get out of bed. Of the patients with placenta praevia or low-lying placenta, 50 were kept in the hospital until after delivery and 16 were discharged and kept under observation as out-patients. Of the latter patients 12 had low-lying placenta and 4 marginal placenta praevia.

Those patients where placenta praevia had been excluded by

radiation hazards. There is mostly an indistinct view of the placental sinuses as they are projected against the pelvic bones. With the aid of the stereoscopic technique described above the localization of the placenta is generally easy by using the films *in frontal projection only* and no *further contrast injection or exposure is required.*

It is possible to reduce the radiation dose even more taking only the second pair of stereoscopic films visualizing the opacified placental sinuses, and by limiting the exposure field to the lower part of the uterus. During the last half of pregnancy the maternal ovaries usually lie outside this field of exposure and so do the foetal gonads in cases of cephalic presentation. If placental sinuses are visible on these films they no doubt, represent the full caudal extension of the placenta. If no placental sinuses are seen the placenta is usually inserted above the lower uterine segment. There are several sources of error to such a judgement however The placenta may be supplied by the ovarian arteries or it may be partially separated from the uterine wall or uterine contractions may have caused absence or reduction in contrast filling of the sinuses. Thus, there are many causes of defective filling of placental sinuses not detectable without examination of the whole uterus. Consequently we are of the opinion that the diagnostic advantage of two pairs of films one pair during the arterial phase and one pair during the venous phase covering the entire uterus, justifies the somewhat increased radiation dose which is still very low

Material

The series includes 179 cases of haemorrhage in late pregnancy Most patients presented with a story of bleeding only additional symptoms such as pain and tenderness being exceptional. In all patients with haemorrhage an internal examination was carried out in the hospital to rule out local lesions in the vagina or cervix causing bleeding.

The radiologic examination was performed in the second trimester of pregnancy in 38 cases and in the third trimester in 141 cases. The examination revealed a *normal implantation of* placenta in 111 cases and placenta praevia or low-lying placenta

in 66 cases. In two cases the placental site could not be definitely determined radiologically. Both were full-term pregnancies where the uterine contractions caused unsatisfactory filling of the placental sinuses. The clinical course suggested a normal implantation of placenta in both cases.

The cases of placenta prævia or low-lying placenta were classified as

Low-lying placenta	26 cases
Marginal placenta prævia	30 cases
Total placenta prævia	10 cases

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Treatment

All patients with diagnosed placenta prævia or low-lying placenta were treated expectantly until the fœtus was considered viable or until severe hæmorrhages forced interruption of the pregnancy. All the patients were kept in bed at the hospital until the bleeding had ceased. If no further vaginal bleeding occurred within a week they were allowed to get out of bed. Of the patients with placenta prævia or low-lying placenta, 50 were kept in the hospital until after delivery and 16 were discharged and kept under observation as out-patients. Of the latter patients 12 had low-lying placenta and 4 marginal placenta prævia.

Those patients where placenta prævia had been excluded by

Table I. *Method of Delivery in Cases of Placenta Prævia*

Position of Placenta	Spontaneous Delivery	Cæsarean Section	Vacuum Extraction
Low-lying placenta	20	5	1
Marginal placenta prævia	13	17	
Total placenta prævia	1	9	
Total	34	31	1

means of arteriography were also kept in bed at the hospital until vaginal bleeding had stopped. Of these patients 71 were later discharged and kept under observation. 42 cases were kept in the hospital until after delivery mainly because they were near term.

Method of delivery

Our method of delivery in cases of placenta prævia does not follow any fast rule but each case is considered individually. Cæsarean section is carried out if the bleeding is profuse and the uterine os is closed or only slightly open. Similarly section is performed in all cases of total placenta prævia. The method of delivery for the cases of placenta prævia and low-lying placenta is shown in Table I.

In one case classified as total placenta prævia but delivered vaginally the radiologic examination showed normal implantation of the placenta but a succenturiate lobe of placenta covering the internal os. This lobe was expelled with moderate hæmorrhage and after that the vaginal delivery was uneventful.

The frequency of Cæsarean section in cases of placenta prævia and low lying placenta was 47 per cent. Of the cases with normal implantation of the placenta 108 were delivered vaginally, 2 by Cæsarean section and 3 by means of vacuum extraction.

Results

There was one maternal death among the cases of placenta prævia. The cause of death was amniotic fluid embolism after vaginal delivery induced by syntocinon infusion.

There were 67 children (1 pair of twins) from mothers with placenta praevia or low-lying placenta 42 boys and 25 girls. This difference is statistically significant. The mothers with normal implantation of placenta had a total of 114 children (1 pair of twins) 62 boys and 52 girls. The prematurity rate was about the same in both groups 33 and 31 per cent respectively The perinatal mortality rate was 13 per cent in cases of placenta praevia or low-lying placenta and 10 per cent in cases with a normal position of the placenta. The difference is not significant. The frequency of congenital malformations was the same in both groups (7 per cent)

The causes of perinatal death in cases of placenta praevia and low-lying placenta were

Immaturity	3 cases
Idiopathic respiratory distress	2 cases
Severe malformation	1 case
Rupture of the tentorium cerebelli	1 case
Diabetic embryopathy	1 case
Death associated with maternal death	1 case

Among the children of mothers with normal implantation of the placenta the causes of perinatal death were immaturity in 11 cases and severe malformation in one case.

Complications of arteriography

In this series there were no severe effects from the radiologic examination. In some cases small haematomas developed at the site of puncture but in no case was any special treatment required.

In 26 cases labour commenced within 48 hours after the arteriography In no case was it thought that arteriography had induced the labour Eleven cases were full time pregnancies, and the others were already in labour prior to the examination.

In no instance could any ill effects on the foetus be ascribed to the arteriographic examination.

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Discussion

Our clinical methods for the diagnosis of placenta prævia are associated with considerable sources of error In recent years different radiologic methods have been used as an aid to diag nosis Some of these methods e g soft tissue radiography and cystography are reliable only if performed during the last month of pregnancy Soft tissue radiography is of limited value in cases of polyhydramnios placenta membranacea and succenturiate placenta and involves a high radiation dose especially to the foetus In addition cystography is only useful in cases of cephalic presentation.

A placenta prævia is often thin and covers a large surface area and even if seen in the upper uterine segment it may because of its unusual size extend into the lower segment. The first symptoms of placenta prævia often appear early in the last trimester of pregnancy In this series the hæmorrhage had already occurred in the second trimester in 38 cases. By means of arteriography the placental sinuses can be visualized by the end of the first trimester of pregnancy and the method is independant of foetal presentation, polyhydramnios and of the position, type and size of the placenta.

The use of a stereo-roentgen technique involves many advantages. It makes it possible to localize the placenta to the posterior or anterior uterine wall in cases where the intervillous spaces are projected over the true pelvis and, consequently saves another injection of contrast medium and a lateral exposure of the pelvis with accompanying discomfort and radiation hazards to the patient. Arteriography with stereoscopic technique is thus the most reliable method to get an early and correct diagnosis of placenta prævia in cases of antepartum hæmorrhage with a minimum of risk to mother and foetus.

An early diagnosis of placenta prævia is essential for the correct handling of the patient and, consequently for the prognosis of mother and child. If placenta prævia is diagnosed, the patient must remain in hospital until after delivery with all the facilities of a modern department available. The great accuracy of local izing the placenta is also of importance for planning the treat ment and may contribute to reducing the frequency of Cæsarean

section in cases of placenta prævia without jeopardizing the life of mother and fœtus. According to modern authors the incidence of Cesarean section in cases of placenta prævia nowadays lies between 60 and 90 per cent. In this series section was performed in only 47 per cent.

With great accuracy in the diagnosis of placenta prævia and under ideal conditions for the treatment maternal mortality is virtually always avoidable. In the present series the only case of maternal death was due to amniotic fluid embolism.

The figures for perinatal mortality in cases of placenta prævia presented by different authors are difficult to compare as they depend upon the accuracy of diagnosis, classification of placenta prævia and different distinctions between mature premature and immature infants Expectant treatment combined with a high incidence of Cesarean section seems to have improved the prognosis for the child. The best results concerning perinatal mortality have been published from England and the U.S.A. In a series of 425 cases of placenta prævia Macafee (1962) reported a perinatal mortality rate of 13.1 per cent. The incidence of Cesarean section was about 75 per cent. In the present series the perinatal mortality rate was about the same (13 per cent) despite the low incidence of Cesarean section (47 per cent) This low perinatal mortality rate combined with a low incidence of Cesarean section is no doubt the result of the great accuracy in localizing the placenta.

It has been said that the exclusion of placenta prævia saves many a hospital bed and the patient and doctor unnecessary anxiety (Brink, 1960) The risks the mother and foetus run in placenta prævia are obvious but it is not generally recognized that the baby runs about the same risk when the source of bleeding is not determinable by existing methods. This fact has been pointed out by Murdoch and Foulkes (1952) who found a foetal loss of 10.3 per cent in this group of antepartum hæmorrhages. This figure is in good agreement with the results in the present series, showing a perinatal mortality rate of 10 per cent in cases where placenta prævia had been excluded. This observation should encourage greater attention in the future in this unexplained group of antepartum hæmorrhages. The main

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An early diagnosis of placenta prævia is essential for the cor rect handling of the patient and, consequently for the prognosis of mother and child. If placenta prævia is diagnosed, the patient must remain in hospital until after delivery with all the facilities of a modern department available. The great accuracy of local izing the placenta is also of importance for planning the treat ment and may contribute to reducing the frequency of Cæsarean

Jansson, Inge Acta obst. et gynec. scandinav. 44, 88, 1966
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EFFECT OF DESAMINO OXYTOCIN ON THE HUMAN PREGNANT UTERUS AT TERM

BY

INGE JANSSON

Oxytocin was first synthesized in 1953 by du Vigneaud *et al* It is a polypeptide containing eight different amino acids. Since then a substantial number of related polypeptides have been synthesized and tested pharmacologically (Boissonas, Guttman, Berde and Konzett, 1961) It has been shown, among other things, that the biological activity of oxytocin is lost if the disulphide bridge between the two cysteine residues is broken. On the other hand, the free amino group of the cysteine residue in position 1 does not seem to be required to activate oxytocin. If hydrogen is substituted for this amino group the cysteine residue is converted into a mercaptopropionic acid residue, and oxytocin becomes desamino-oxytocin (Fig. 1) This compound also was first synthesized by du Vigneaud *et al.* (1960)

The pharmacological properties of desamino-oxytocin have been investigated in animal experiments by Chan and du Vigneaud (1962) and by Berde and Saameli (1963) They found that desamino-oxytocin had twice the effect of oxytocin on the cat uterus in vivo and was one and a half times as effective on rat uterus in vitro The effect on the chicken blood pressure was also about one and a half times higher than that of oxytocin. However the vasopressor activity when determined on rats, seemed to be lower than that for oxytocin. Information with regard to its antidiuretic effect differs Berde and Saameli (1963) found a lower antidiuretic activity for desamino-

cause of perinatal death in this group was immaturity It seems probable that many of the premature births could be prevented by providing the same care for these patients as is done in cases of placenta prævia

As a matter of curiosity it may be mentioned that among children to mothers with placenta prævia and low-lying placenta there were more boys than girls The significance of this finding is obscure and we have not been able to find any similar observation published in earlier literature.

SUMMARY

One hundred and seventynine cases of antepartum hæmorrhage were examined by means of arteriography using a stereo-rœntgen method. The technique and the advantages of the method are discussed.

In 66 cases placenta prævia or low lying placenta was diagnosed. There were no noteworthy complications from the examination.

There was one maternal death among the cases of placenta prævia The cause of death was amniotic fluid embolism. The perinatal mortality rate was 13 per cent among the cases of placenta prævia or low-lying placenta. The incidence of Cæsarean section was 47 per cent. Among the cases with a normal placental site the perinatal mortality rate was 10 per cent. The difference is not significant The implications of these findings are discussed. A plea is being made for greater attention to the prenatal care of this group of patients with unexplained antepartum hæmorrhages.

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Berde and Saameli (1963) studied also the effect of desamino-oxytocin on the human puerperal uterus. By means of external tocography they recorded the duration of increase in tonus and found that the activity of desamino-oxytocin was twice that of oxytocin. Recently Embrey (1965) has studied the effects of desamino-oxytocin on the human pregnant uterus using both external and internal tocography No qualitative difference was found between the oxytocic effect of desamino-oxytocin and that of oxytocin. There was, however a quantitative difference. Measured in accordance with the duration of the hypertonus following a single injection, desamino-oxytocin had an oxytocic activity more than twice that of the parent hormone.

Methods

Desamino-oxytocin, under the name of ODA 914, was supplied for clinical trials by the courtesy of the Sandoz laboratories The preparation is obtainable in ampoules each containing 2 units ("human i.e in terms of its effect on human puerperal uterus) or 1.4 units (chicken i.e effect on chicken blood pressure 1 μg = 1 IU human = 0 7 IU chicken)

The effect of ODA 914, injected intravenously on the human pregnant uterus at term, was compared with that of oxytocin (Syntocinon Sandoz) Our clinic has extensive experience of Syntocinon used in the Smyth oxytocin sensitivity test (OST) before induction of labour with Syntocinon by intravenous infusion. Consequently it was thought appropriate to try ODA 914 in the same way whilst also subjecting each patient to trials with Syntocinon and then comparing the results. The trials were performed on a series of patients where induction of labour was indicated. The uterine activity was recorded with the Malmström-Thorén external tocograph.

The oxytocin sensitivity test was performed as originally described by Smyth (1955) After recording any uterine activity under resting conditions for 10–15 minutes a solution of Syntocinon or ODA 914, containing 0.01 IU/ml was injected intravenously at the rate of 1 ml every minute, up to a total dose of 0 10 IU A uterine contraction, producing an increase in uterine

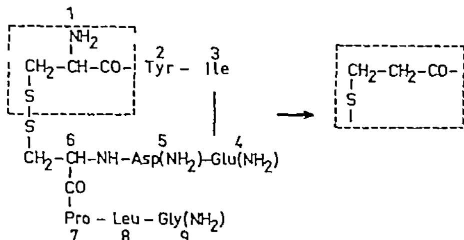


Fig. 1 Schematic representation of the conversion of oxytocin into desamino-oxytocin. the cysteine residue in position 1 becomes a mercaptopropionic acid residue. The abbreviations stand for the amino acid residues tyrosine, isoleucine, glutamic acid aspartic acid, proline, leucine and glycine.

oxytocin as compared with that for oxytocin, whereas Chan and du Vigneaud (1962) observed that it was three times more active than oxytocin.

Human pregnancy serum inactivates oxytocin. This is attributed to an enzyme called oxytocinase. According to Tuppy and Nesvadba (1957) this enzyme is an aminopeptidase which opens the pentapeptide ring of oxytocin between the cysteine and tyrosine residues. Golubow Chan and du Vigneaud (1963) showed that human pregnancy serum does not have this effect on desamino-oxytocin. The avian depressor activity of this compound remained unaltered after incubation with human pregnancy serum. Thus the lack of a free amino group renders desamino-oxytocin resistant to attack by pregnancy serum. This may explain the higher biological activity of desamino-oxytocin. However it must be borne in mind, as pointed out by Embrey (1965) that oxytocinase is probably not the only or even the most important clearing factor of oxytocin *in vivo*

in less than 12 hours with oxytocin infusion alone (Jansson, 1964)

Consequently the patients were divided into three groups patients positive with 0.01–0.04 IU those positive with 0.05–0 10 IU and those who did not respond at all to the trial dose called negatives. In this investigation the condition of the cervix was not regarded as of essential interest and was excluded. Table I shows the distribution of test results

Table I. *Distribution of Test Results with Syntocinon and ODA 914 in 8 Cases*

		ODA 9 4		
		0 –0 04 IU	0.05–0 0 IU	Neg
Syntocinon	0.0 –0.04 IU	1	9	6
	0.05–0 10 IU	4	12	7
	Neg.			29

The series is too small to permit any conclusions being based on a comparison between the individual groups. If the two positive groups are taken together it is obvious that there is a definite correlation between the results obtained with Syntocinon and those obtained with ODA. Out of 49 patients positive with 0.01–0.10 IU of Syntocinon, 36 also had a positive response with ODA. Of the 33 patients who did not respond to Syntocinon, 29 were negative also with ODA.

With regard to the prognostic significance of the trials about the same results were obtained for both Syntocinon and ODA. Out of 26 patients positive with 0.01–0.04 IU of Syntocinon, 17 (65 per cent) were delivered spontaneously within 24 hours, whereas 10 out of 17 (59 per cent) who were responsive to the same doses of ODA, were delivered within 24 hours For the other four groups the results were about the same slightly over 40 per cent being delivered within 24 hours It is necessary to stress, as previously mentioned, that cervical ripeness must be regarded as increasing the prognostic values of the tests. Furthermore, labour was almost invariably induced with Syntocinon infusion. The ODA test might have proved a more reliable guide

pressure of not less than 20 mm of Hg was regarded as a positive response. There was an interval of not less than 30 minutes between the Syntocinon and the ODA tests. In half of the tests, Syntocinon was given initially whereas in the other half ODA was used first. The tests arranged in this way afforded a more reliable comparison between the results because a certain increase in uterine sensitivity to oxytocin can be expected after the first test. The uterine sensitivity was noted in each case *i e* the dose of ODA or of Syntocinon required to produce a uterine contraction of sufficient strength. The appearance and duration of the individual contractions were also studied.

After testing the patient, labour was induced, usually with Syntocinon infusion but, in a few cases with ODA. Uterine activity was recorded continuously by external tocography The uterine contraction pattern was studied and the time until delivery noted. Primary amniotomy was not performed.

Material

Eighty two pregnant women at term were tested with both Syntocinon and ODA 914 42 primigravidæ and 40 multigravidæ Tests with ODA only were made on another 10 patients In three of these patients it was impossible to make a Syntocinon test, because active spontaneous labour commenced after the ODA test. Another five patients were given an ODA infusion without any preceding test Thus the effect of desamino-oxytocin on the pregnant uterus was investigated in 97 patients. Eleven of these were given an intravenous infusion of ODA 914

Results

As was shown earlier (Smyth 1957 Husslein and Baumgarten, 1961 Jansson 1964 and others) there is a correlation between the test response *i e* the uterine sensitivity to oxytocin, and the outcome of induction of labour If the uterus is sensitive to 0.04 IU or less it is highly probable that labour will begin a short time after either amniotomy or infusion of oxytocin alone. With a ripe cervix and a positive test to 0.01–0.04 IU more than 80 per cent of the patients will be delivered

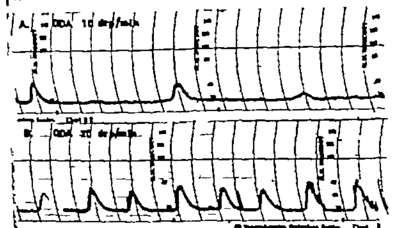


Fig. 3. Patient, aged 20, gravida 2, para 1. Stimulation of labour with desamino-oxytocin. A. drops/min: weak contractions with intervals of 7 minutes (1 minute between each vertical line on paper) B. 20 drops/min: good contractions with min intervals. Concentration of ODA infusion 4 IU in 500 ml 5.5 % glucose. Delivery in less than hours.

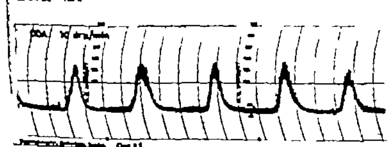


Fig. 4. Patient, aged gravida 2, para 1 Induction of labour with ODA infusion after preceding test. Strong regular contractions with drops/min. Delivery in less than hours.

A hypertonic contraction lasting several minutes was observed in 9 patients after various doses of ODA. Five of these patients also had the same pattern of uterine activity in the Syntocinon tests (Figs. 6 7)

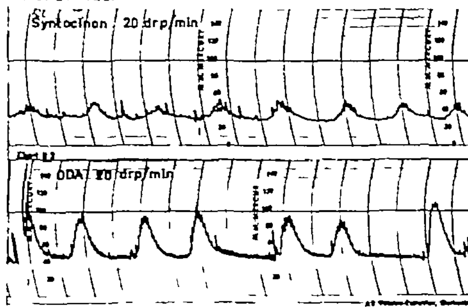


Fig. 2. Patient, aged 25 gravida 1 Induction of labour with Syntocinon (5 IU in 500 ml 5.5 % glucose) followed by ODA 914 (4 IU in 500 ml 5.5 % glucose) Stronger contractions with the same infusion rate of ODA. Unripe cervix. Induction trial unsuccessful.

to the outcome of induction if it had been used instead of Syntocinon for the subsequent drip infusion. Out of the 11 patients who were given ODA by intravenous infusion, 7 were delivered in less than 24 hours irrespective of tests and cervical ripeness. In one of these patients the preceding Syntocinon infusion had had no effect. In two other patients who were first given a Syntocinon infusion without success, the following infusion of ODA was likewise unsuccessful. In one of these patients ODA produced stronger and more regular contractions than did Syntocinon (Fig 2) but the unripe cervix did not dilate and the induction trial had to be discontinued.

Uterine contractile response to ODA

In most patients with a positive response to ODA, the contraction curve was normal and its appearance was the same as that in spontaneous or in Syntocinon induced labour (Figs. 3 4 5)

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A. ODA-test

1 2 3

B. Syntocinon test

1 2 3 4

C. Syntocinon infusion

Fig. 6. Patient, aged 33, gravida ODA test positive to 0.03 IU (A) Syntocinon test positive to 0.04 IU (B) Hypertonus lasting 6–7 minutes after both tests. Same contraction pattern recorded on Syntocinon infusion 20 drops/min. (C) Concentration of Syntocinon 5 IU in 500 ml 5.5 % glucose. Each arrow indicates the injection of 0.0 IU of Syntocinon or ODA 914

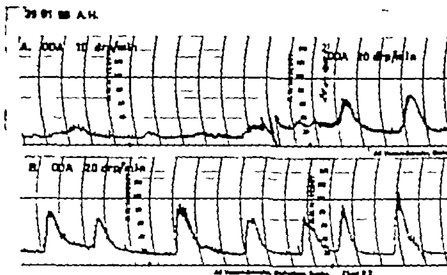


Fig. 5 Patient, aged 26 gravida 1 Induction of labour with ODA. A. 10 drops/min. no effect. B. 20 drops/min. strong effective labour Delivery in less than 12 hours The rise in basic level of the curve is caused by the patient changing from a supine to a lateral position.

In one patient prolonged hypertonic contractions were recorded during spontaneous labour These contractions began two days after the tests (Fig. 7) This is of special clinical and theoretical interest. The pattern of contraction was obviously not dependent upon the drug chosen to induce labour but was perhaps inherent in the uterus itself

One patient had protracted hypertonic contractions in both the test and the infusion with Syntocinon (Fig. 6) In four other patients uterine hypertonicity was observed during Syntocinon infusion, which made it necessary to discontinue the trial induction. In this series 92 Syntocinon infusions were given. The dose was 5 IU of Syntocinon in 500 ml of 5 5 per cent glucose with an infusion rate of 10–30 drops/min.

None of the 11 patients who were given an *intravenous infusion* of ODA showed any tendency to hypertonus. The dose used was 4 IU in 500 ml of 5 5 per cent glucose with an infusion rate of 10–30 drops/min.

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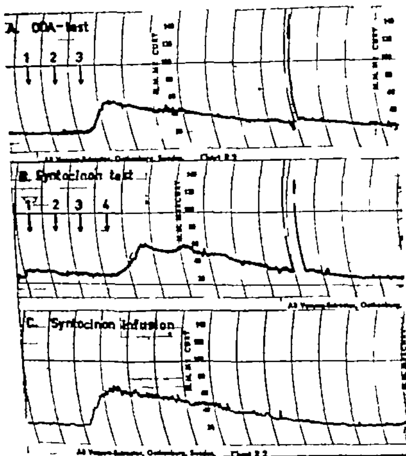


Fig. 6. Patient, aged 33, gravida 1. ODA test positive to 0.03 IU (A). Syntocinon test positive to 0.04 IU (B). Hypertonus lasting 6–7 minutes after both tests. Same contraction pattern recorded on Syntocinon infusion 20 drops/min. (C) Concentration of Syntocinon 5 IU in 500 ml 5.5 % glucose.
Each arrow indicates the injection of 0 IU of Syntocinon or ODA 914.

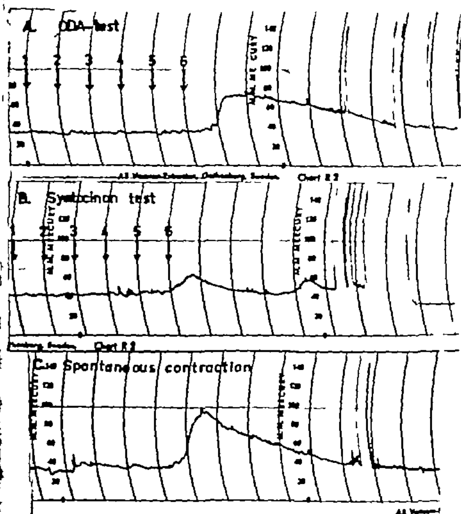


Fig 7 Patient, aged 24 gravida 2 para 1 Intrauterine death of foetus in previous pregnancy ODA test (A) and Syntocinon test (B) pos. to 0.06 IU Long contraction after ODA test and slight hypertonicity also after Syntocinon. Strong contraction lasting 5 minutes in spontaneous labour two days later (C) Slowing of foetal heart rate during hypertonus (Interruptions in recording indicate listening to foetal heart sounds) Discoloration of amniotic fluid on amniotomy Caesarean section resulting in living child.

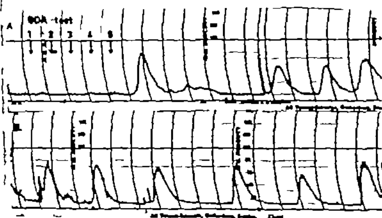


Fig. 8. Patient, aged 20, gravida A. ODA test positive to 05 IU B. Spontaneous labour after test. No infusion.

Spontaneous labour after tests

Following oxytocin sensitivity tests a number of spontaneous uterine contractions are often observed. These contractions are mostly weak and irregular The term spontaneous labour is used here to designate strong, regular contractions after the test for not less than 30 minutes (Fig. 8) These occurred 15 times after 92 ODA tests as compared with 5 times after 91 Syntocinon tests. In one of the patients whose labour started spontaneously after the ODA test, delivery took place in 3 1/2 hours without an oxytocin infusion.

The child

Two children were dead before the induction of labour One was an anencephalic monster and the other a macerated second twin. No child was lost during or after delivery There were four premature infants three of whom were twins. There was no morbidity attributable to the induction of labour among the live born children.

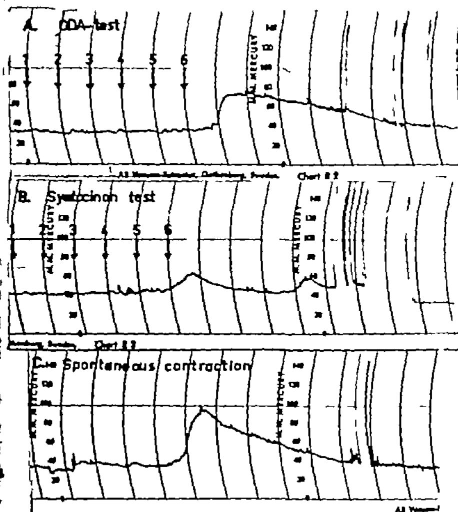


Fig. 7 Patient, aged 24 gravida 2 para 1 Intrauterine death of foetus in previous pregnancy ODA test (A) and Syntocinon test (B) pos. to 0.06 IU Long contraction after ODA test and slight hypertonicity also after Syntocinon. Strong contraction lasting 5 minutes in spontaneous labour two days later (C) Slowing of foetal heart rate during hypertonus (Interruptions in recording indicate listening to foetal heart sounds.) Discoloration of amniotic fluid on amniotomy Caesarean section resulting in living child.

patient who was irresponsive to the oxytocin infusion, delivery was rapid after the infusion of ODA. In two other patients the ODA infusion was as unsuccessful as the preceding oxytocin infusion, even if a stronger and more regular contraction pattern was observed when ODA was used. There are, of course, many factors which determine whether or not delivery will occur within a certain time, *e.g.* as previously mentioned, the condition of the cervix. Furthermore it is very difficult to base an evaluation on a comparison of individual cases, since, in clinical experience uterine sensitivity to oxytocin can change rapidly. The results of a larger series of ODA infusions for induction of labour must be collated before a fair comparison with oxytocin can be made.

As has been emphasized by other investigators (Berde and Saameli, 1963 Embrey 1965) there is, however a quantitative difference. The ODA preparation used was standardized on oxytocin applied to the puerperal uterus, and was found to be twice as active as oxytocin by weight (Berde *et al.* 1963). Moreover in the present study a lower concentration, 8 mU/ml of desamino-oxytocin was used as compared with 10 mU/ml of oxytocin, and induced effective labour at the same, or even at a lower infusion rate. In other words, in intravenous infusion, desamino-oxytocin was more than twice as active as oxytocin in inducing effective labour.

SUMMARY

Desamino-oxytocin is a new analogue of oxytocin. From a chemical point of view it differs from oxytocin only in the lack of an amino group in position 1. In animal experiments this new compound has shown a higher oxytocic, avian depressor and, possibly also a higher antidiuretic activity than oxytocin. Its higher potency may be due to the fact that it is not broken down by oxytocinase.

The present study is concerned with the comparative effects of desamino-oxytocin and oxytocin on the human pregnant uterus at term, when using the Smyth oxytocin sensitivity test and external tocography. No qualitative difference was found be-

Intrauterine asphyxia

Slow and irregular foetal heart sounds were heard in five cases three in connection with Syntocinon infusion, and two during contractions lasting 8–9 minutes in the ODA tests The foetal heart sounds rapidly became normal when the uterine pressure was again at basic level. Meconium staining of the amniotic fluid was noted in four cases In two of these labour had been induced with Syntocinon the other two were those previously mentioned in connection with hypertonus and the tracings are shown in Figs 6 and 7 No signs of extrauterine asphyxia appeared in any of the children.

Discussion

When compared unit for unit desamino-oxytocin does not seem to differ from oxytocin in its effect on the pregnant uterus at term. Its pattern of uterine contraction is the same as that observed in oxytocin induced and in spontaneous labour Since desamino-oxytocin is not affected by oxytocinase, prolonged action may perhaps be expected. In a few instances hypertonic contractions were observed which lasted several minutes and were complicated by the slowing of the foetal heart rate. This however does not seem to be more common with desamino-oxytocin than with oxytocin. It also occurs in spontaneous labour

Thus both a single uterine contraction and a series of contractions are the same whether produced by oxytocin or by desamino-oxytocin. There may however be a qualitative difference in another respect The occurrence of spontaneous labour following the ODA tests was three times more frequent than that after the Syntocinon tests This may be owing to the prolonged action of desamino-oxytocin, but it should be pointed out that the series is too small to exclude the possibility that this phenomenon is due to a difference in uterine sensitivity

Whether desamino-oxytocin is more effective than oxytocin in starting labour cannot be determined by this study Rapid delivery took place in 7 out of 11 patients as a result of the infusion of ODA, which is about the same frequency as that for oxytocin, if cervical ripeness is not taken into account. In one

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MONOAMNIOTIC TWINS

BY

MARNER SIMONSEN

Monoamniotic twin pregnancy is the condition where twins are contained in a single amniotic sac.

As this condition is relatively rare, the purpose of the present paper is to give a short review of the literature to show the increased danger to the foetuses, and to report two additional cases of monoamniotic twin pregnancies occurring in the gynaecologico-obstetric department of the Copenhagen County Hospital in Glostrup during the last 4 years.

History

The first cases of twins contained in one sac were reported by Boccalini (1612) Jacob von Beck (1648) and Viardel (1671)

In the eighteenth century only one case of monoamniotic twin pregnancy was described.

Between 1805 and 1903, 71 cases were collected by Alfieri (1903)

In the following years additional cases were reported by Quigley (1935) Craig (1957) and Raphael (1961) thus bringing the total number of monoamniotic twin pregnancies recorded in the world literature up to 183.

The correct figure is undoubtedly higher as some European cases are not included in the American survey of the literature.

So far only 8 cases of monoamniotic twin pregnancies have been reported in the Scandinavian literature. The first case was reported by Pers (1922) but no details were given.

tween oxytocin and desamino-oxytocin. In a few cases hyper tonic contractions lasting some minutes occurred after intra venous injection of desamino-oxytocin, after oxytocin injection, and also in spontaneous labour Spontaneous labour occurred more often after the desamino-oxytocin test alone, than follow ing the oxytocin test possibly indicating the prolonged action of ODA in this connection.

In 11 cases intravenous infusion of desamino-oxytocin was used to induce labour Active labour was produced by a dose less than half by weight that required for oxytocin. No com plications occurred in either mother or child when treated with an intravenous infusion of desamino-oxytocin.

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Simon (1926) reported one case but gave very little information except a description of the placenta and discussion of various theories of the ætiology of the condition.

Guldberg (1938) described one case of monoamniotic twins, one twin being a normal female, while the other was a female pseudohermaphrodite. The latter who died aged 5 days had various malformations of the digestive tract and the urinary system in addition to the genital malformation. On post-mortem examination ovarian tissue was demonstrated, but no testicular tissue

Henrichsen (1946) reported one case of monoamniotic twins delivered in the eighth month of gestation. Both were still born owing to knotted cords.

Trolle (1958) reported 4 cases of monoamniotic twins collected during 2 1/2 years from among 5100 deliveries. Of these there was double survival in only one case. In one case both twins died shortly after delivery and in the third case one twin died neonatally In the last case both were still-born.

Ætiology

The type of uniovular twinning is dependent on the time at which the fertilized ovum divides.

Dichorionic, diamniotic uniovular twins result from division of the morulla before any cellular differentiation and ability to form chorionic tissue has developed. (Fig 1)

It is believed that the inductive force in the formation of the amnion is the presence of the embryo for no normal ovum has been described where an amniotic sac was present prior to the formation of ectoderm and endoderm.

The amnion develops between the seventh and the thirteenth day after fertilization. If the split in the germinal disc occurs prior to that time both embryonic plates will form their own amnion resulting in monochorionic diamniotic twins If the split in the germinal disc occurs sometime between the seventh and the thirteenth day and before the axial arrangement can be observed, the result will be monoamniotic uniovular twins

If the split occurs after the thirteenth day it will be incomplete since an axial arrangement has already been formed. This

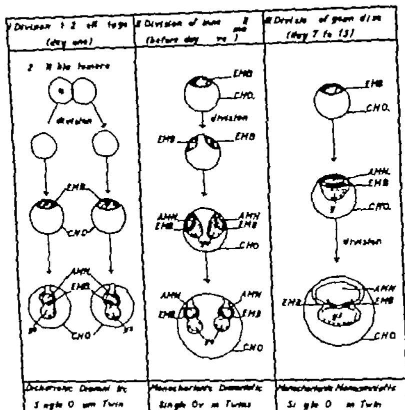


Fig. Three mechanisms of single ovum twinning diagrammatically illustrated (Modified after G. W. Corner 1. Craig and Salerno)

will result in a certain degree of fusion of the embryos contained in a single amniotic sac—so called conjoined or Siamese twins.

About 25–30 per cent of monozygotic twins are dichorionic, diamniotic. Consequently the demonstration of four tissue layers (two chorions and two amnions) between the amniotic cavities is not proof that twins are dizygotic.

On the other hand a two-layered septum does not prove that the twins are monozygotic, as the interjacent chorionic sheets may be so thin that they can only be demonstrated microscopically

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If the split occurs after the thirteenth day it will be incomplete since an axial arrangement has already been formed. This

Table II. *Increase in Double Survival of Monoamniotic Twins as Reported in the World Literature*

Author	and Year	No. Cases	Double Survival	
			No.	%
Quigley	935	99	17	16
King	1952	148	30	20
Craig	957	66	40	24
Raphael	196	83	51	28
Weininger	1962	94	59	30
935 to 1962		85	42	49

Table III.

Reported Scandinavian Cases						
Author	and Year	Cases	Double Survival	Single Survival	Double Stillbirth or Perinatal Death	Cord Knots
Pers	922		?	?	?	?
Simon	926		?	?	?	?
Goldberg	938					
Henrichsen	946					1
Trolle	958	4		1		1
Present study	965					1

Foetal survival

It is commonly stated that in multiple pregnancy greater perinatal risk is involved.

Newman (1942) and Morison (1952) stated that the risk of still-birth is 3 to 4 times greater in deliveries of dizygotic twins, and 6 to 7 times greater in deliveries of monozygotic twins.

In monoamniotic twin pregnancies the risk is even greater

Quigley (1935) reviewed the earlier literature and found that the chance of double live birth was only 18 per cent, and that the total foetal mortality was 68 per cent. (Table II.)

Raphael (1961) in reviewing the world literature found the double survival rate to be 28 per cent. However in the cases of monoamniotic twin pregnancy reported since 1935, the double survival rate was about 50 per cent.

Table I *Comparison of Incidence of Monoamniotic Twin Pregnancy*

Author	Ratio of Monoamniotic Twins to Total Pregnancies	Ratio of Monoamniotic Twins to Twin Deliveries
Ahlefeld	1 16,000	1 169
Aigner	1 7 000	
Rosenberg	1 60,000	1 700
Müller	1 6 000	1 70
Alfieri		1 256
Acosta-Sison	1 93,734	1 661
Librach et Terin	1 4,000	1 65
Raphael	1 16,000	1 165
Trolle	1 1 700	1 33
Leroy	1 16 000	
Wensinger	1 3,071	
Coplerud	1 40,000	1 400
Present study	1 1 650	1 52

The diagnosis of dizygotic twins is only certain where the infants are of different sex.

In all other cases only a later detailed comparison may decide the question.

Incidence

The incidence of monoamniotic twins given in the world literature differs widely (Table I)

Obviously the true incidence of monoamniotic twin pregnancies cannot be calculated with accuracy unless obstetricians bear this possibility in mind at all twin deliveries and report all such cases.

In the obstetric department of the Copenhagen County Hospital in Glostrup 3300 deliveries took place during the four-year-period 1961–1964 Of these 104 were twin deliveries, including 2 cases of monoamniotic twins.

The high incidence of twin deliveries (1 33) is due to the fact that the hospital only receives abnormal cases.

The incidence of monoamniotic twin delivery was one set in every 52 twin deliveries



Fig. 2. Placenta of Case

Simultaneously two intertwined cords were observed in the vagina tied in knot.

By vaginal exploration head was felt, but no second amniotic sac.

The delivery of the second twin was accelerated by expression, and 5 minutes after the delivery of the first twin live boy was delivered, weight 2950 g

5 minutes later the placenta was delivered spontaneously Both cords were inserted in the middle of the placenta, two cm. apart. Examination of the membranes revealed one amnion and one chorion, and, as mentioned, knot ting of the cords.

) J nr 4509 12 M

9-years-old gravida para

The pregnancy had progressed quite normally and the patient was admitted in labour with ruptured membranes 7 weeks before the expected date of confinement.

Shortly after admission she was delivered of live boy weighing 1350 g in cephalic presentation.

Examination revealed foetus in vertex presentation and absence of second bag of waters.

In the cases reported in Scandinavia similar rates are found. (Table III) Since 1958 6 cases have been reported with a 50 per cent double survival rate.

The poor fœtal survival in monoamniotic twin pregnancies is partly due to the interference with fœtal circulation as a result of the twisting and knotting of the umbilical cords. The knots may be tightened during labour resulting in reduction or complete interruption of the blood circulation to the fœtuses.

Another cause of higher perinatal mortality is the tendence to prematurity common in plural births.

The majority of the monoamniotic twins mentioned in the world literature have been born between the 34th and 37th weeks of gestation.

Incidence of anomalies

It is remarkable that severe congenital malformations are reported in about 10 per cent of the monoamniotic twins in the world literature. In most cases only one twin is malformed, thus excluding a direct genetic factor or a generalized intrauterine condition, as this would affect both fœtuses equally This may be explained by a local defect causing reduced vitality of different embryonic tissues (Streeter 1930) This local defect may be caused by the splitting in monoamniotic twins happening relatively late and at a more advanced stage of the development of the germ disc, thereby causing disturbance of the growth or reducing the vitality of the embryonic tissue, thus increasing the risk of localized congenital anomaly e g anencephaly in monoamniotic twins (Pedlov Corner Stolk)

Additional cases

1) J.nr 460426 E.L.
18-years-old, gravida 1 para 0.
The mother of the patient is a twin
During pregnancy no abnormalities were observed except for a rather excessive increase in weight of 17 kg.

The patient was admitted on the expected day of confinement. Labour had then begun.

After 3 hours of labour she delivered a live boy weighing 2950 g



Fig. 2. Placenta of Case

Simultaneously two intertwined cords were observed in the vagina tied in knot.

By vaginal exploration a head was felt, but no second amniotic sac.

The delivery of the second twin was accelerated by expression, and 5 minutes after the delivery of the first twin live boy was delivered, weight 1950 g.

15 minutes later the placenta was delivered spontaneously. Both cords were inserted in the middle of the placenta, two cm. apart. Examination of the membranes revealed one amnion and one chorion, and, as mentioned, knotting of the cords.

) J.nr. 4509/2 M.e.

9-years-old gravida para

The pregnancy had progressed quite normally and the patient was admitted in labour with ruptured membranes 7 weeks before the expected date of confinement.

Shortly after admission she was delivered of live boy weighing 350 g in cephalic presentation.

Examination revealed a foetus in vertex presentation and absence of second bag of waters.



Fig 3 Placenta of Case 2.

5 minutes after the delivery of the first twin the second twin was delivered by expression. It, too was a male in good condition weighing 2300 g

15 minutes later the placenta was delivered spontaneously It consisted of a normal placenta without infarctions, one amnion and one chorion. The cords were inserted centrally 6 cm apart. There was no knotting or twisting of the cords

Discussion

The incidence of monoamniotic twin pregnancies is variously reported.

According to Trolle s findings made in the University Clinic of Obstetrics (Rigshospitalet) in Copenhagen, and according to the present study the ratio of monoamniotic twins to the number of twin deliveries is 1 40.

Trolle s incidence and that found by the author are surprisingly similar probably owing to the particular attention paid to this condition.

Most likely almost all detected cases of monoamniotic twins are reported, and the different incidences found may be due to

lack of acquaintance with the condition or to its easily being overlooked.

There is no way of diagnosing this condition prior to delivery

At the time of delivery the diagnosis is made by observing

1) the absence of a second amniotic sac,
2) a possible twisting and knotting of the umbilical cords
3) the condition of the placenta one common placenta without any septum. There may be anastomoses between the vessels of the umbilical cords.

It is important to bear this condition in mind in spite of its relative infrequency since a high perinatal mortality is found in monoamniotic twin delivery owing to the condition of the umbilical cords.

It is therefore important to hasten the delivery of the second twin as much as possible as soon as the diagnosis has been made, as the chances of survival of the second twin are greatly reduced.

SUMMARY

Two cases of monoamniotic twin pregnancies with double survival are reported, thus bringing the number of reported cases in the Scandinavian literature up to 10.

The literature has been reviewed and the aetiology as well as the frequency of monoamniotic twins discussed. The great incidence of anomalies and the highly reduced chances of survival of the twins are mentioned.

The frequency of double survival is now approximately 50 per cent.

The diagnosis is made at delivery of the second twin and is based on the absence of a second amniotic sac or on the twisting and knotting of the cords.

Attention is drawn to the importance of hastening delivery of the second twin, immediately the diagnosis has been made.

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Fig 3 Placenta of Case 2.

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SEQUENTIAL AND COMBINED THERAPY IN ORAL CONTRACEPTION

Mode of Action and Efficiency

BY

JØRGEN KAISER, LEIF WIDE AND CARL GEMZELL

Mode of Action

The mode of action of oral contraceptives is incompletely known. Firstly they do not always block ovulation (Holmes and Mandl 1962 Rice and Wray et al. 1963) and thus, they seem to induce temporary sterility by other means e.g. by bringing about changes in the cervical mucous or in the endometrium. Secondly there are apparently several ways in which some steroids inhibit ovulation. This inhibition may be caused by a direct action on the ovary e.g. by decreasing the sensitivity of it to the follicle stimulating hormone (FSH) (Gemzell, 1962) Another site of action may be a reduction of the release from the pituitary gland of FSH, LH, or both.

In the human, numerous authors have shown that treatment with some steroids causes a decrease in the urinary gonadotrophin excretion (Pincus 1957 Douglas et al. 1960 Buckholz et al 1964 Demol and Ferin, 1964 Lin et al. 1964 Taymor 1964) while others have been unable to demonstrate any such change (Heller 1957 Smith and Albert, 1958 Albert and Smith, 1961 Loraine et al. 1961 McArthur et al. 1961 Brown et al. 1962 Fuchs et al. 1964)

Such divergent findings could be due to the use of different amounts of steroids at different dose levels or to the method

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urine, and one drop of a 2.5 % HCG coated blood cell suspension. The LH activity was expressed in units equivalent to 10–20 units of HCG/litre.

Pregnanediol estimation. During the menstrual cycle, two estimations of urinary pregnanediol were made on each of the thirteen patients. These determinations were done on day 4, 5 or 6 and again on day 20 21 or 22. The method used was that of Goldzieher and Nakamura (1962) Methylene chloride was used instead of chloroform for the extraction (Carstensen, 1962) The urine samples were stored at —20 C until required.

Results

Five pregnancies occurred during the sequential therapy One of these patients suffered from severe and frequent vomiting and discontinued the medication on day 11 of the second tablet cycle, during which she became pregnant. The other four pregnancies were tablet failures. One of the patients had taken the tablets throughout two cycles, but after the second one no withdrawal bleeding occurred. Believing that this was only a case of amenorrhœa, sometimes seen as a consequence of oral contraception, she was advised to start a third course of tablets on the 7th tablet free day A specimen of urine was subsequently collected for pregnanediol determination and when the level was elevated, a pregnancy test was performed, which was positive.

When the first pregnancy was diagnosed, 3 other cases of amenorrhœa following the treatment were immediately investigated and found to be due to pregnancy Two of these women had conceived during the second tablet cycle. The 5th woman who became pregnant had taken tablets regularly for 3 consecutive cycles. On the 20th day she forgot to take the pill. From day 21 a condom was used in addition to the tablets. No bleeding ensued, and a pregnancy was diagnosed. All these patients were examined before the sequential therapy was started and had normal menstrual cycles.

Anovlar® was given to the remaining 66 patients. No pregnancy occurred during the next 227 cycles.

used for the assay of the urinary gonadotrophins This paper deals with hormone analyses of the urine during treatment with two different oral contraceptives administered as sequential or combined therapy The effects of these treatments on the urinary LH and pregnanediol excretion are discussed.

Material and Methods

Patients and clinical follow-up This investigation initially comprised 36 patients mainly university students who had at least one previous pregnancy The treatment began in February 1964 with the following type of sequential therapy 0 05 mg of ethinyl-oestradiol (17 β-ethinyl 17 α-oestradiol) for 20 days, and 5 mg of medroxyprogestrone acetate, Provera[R] (6 α methyl 17 α acetoxyprogestrone) added during the last 10 days The medication started on the 5th day of the menstrual cycle. If the expected bleeding did not occur after the end of the course of tablets, the volunteers were instructed to start another course of tablets 7 days later When, after 4 months pregnancies were observed in some cases the other volunteers were advised to use other contraceptive methods in addition to the tablets during the rest of the cycle. At this time 210 cycles of treatment had been completed. All the patients were then transferred to a treatment of 0.05 mg of ethinyl oestradiol combined with 4 mg of norethisterone acetate, Anovlar[R] (17 α-ethinyl 19-nor testosterone acetate) for 21 days At the time of writing, 227 cycles with Anovlar[R] were completed.

LH Assays The urinary LH excretion was assayed immunologically by a modification of the method described by Wide *et al* (1961) in which the immunological cross reaction between human chorionic gonadotrophin (HCG) and LH was exploited. Formol and tannic acid treated erythrocytes were coated with HCG and agglutinated by rabbit antiserum to human pituitary LH. The preparation of the antisera and the HCG coated blood cells have previously been described in detail (Wide 1962) The reactions were performed in agglutination plates with cavities 16 mm in diameter×8 mm deep To each cavity was added 0 4 ml of various dilutions of the antiserum 0 5 ml undiluted

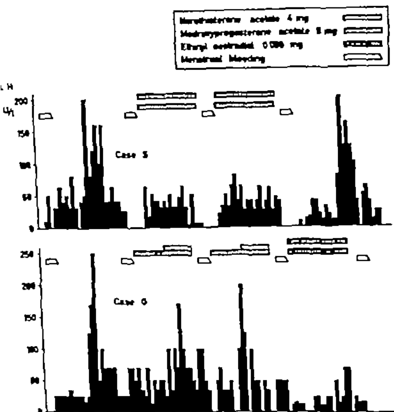


Fig. 2. Urinary LH excretion, as assayed by an immunological method, of two women during cycles without and with hormone therapy. During untreated cycles both patients showed normal excretion pattern. Case S. was treated with Anovlar® during cycles. During these cycles no mid-cycle peak appeared. Case G. was firstly treated with our variant of sequential hormone therapy and no change in the LH excretion was registered. However when the same patient during the subsequent cycle was treated with Anovlar® the high mid-cycle peak of LH failed to appear

Two distinct differences were observed between the two regimes with regard to side effects. The sequential therapy with ethinyl oestradiol and medroxyprogesterone acetate was associated with a high rate of break through bleedings, (18.8 %) Anovlar® caused a pronounced weight gain. However no con

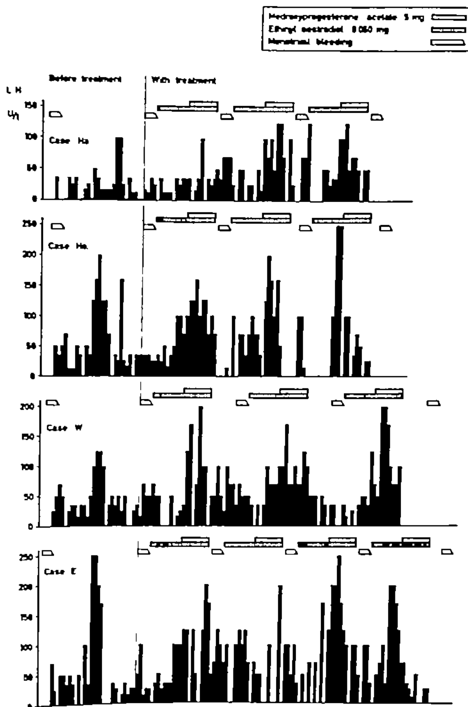


Fig. 1 Urinary LH excretion as assayed by an immunological method, of four women before and during sequential hormone therapy The excretion before treatment shows a normal pattern with a mid-cycle peak. The excretion during sequential hormone therapy (0.05 mg of ethinyl oestradiol for 10 days with 5 mg of medroxyprogesterone acetate added for the next 10 days) did

ment with Anovlar[2] the normal mid-cycle LH peak failed to appear

The excretion of pregnanediol before and during sequential therapy is shown in Fig. 3 During days 4 5 or 6 of all cycles the excretion was below 1.0 mg per 24 hours On days 20 21 or 22 of the control cycles the pregnanediol excretion was elevated (more than 1.0 mg per 24 hours) in 9 out of 13 cycles while in the treated cycles only two out of 28 were above 1 mg

Discussion

Goldzieher *et al.* (1963) introduced the sequential oral con traceptive therapy by using 0.08 mg of the 3-methyl ether of ethinyl oestradiol (mestranol) alone for 15 days with 2 mg of 6-chloro-ene-17-acetoxy-progestrone (chlormadinone) added for the last five days. His initial report based on 938 women for 6314 cycles includes 3 pregnancies of which 2 were considered to be due to patient error In a later publication (Goldzieher et al. 1964) the material had been extended to 1 191 women followed for 11,001 cycles during which 6 pregnancies occurred due to tablet failure. Mears (1964) used the same medication as that of Goldzieher and in March 1964, she reported 2 pregnancies in 150 patients for 930 cycles one of which may have been due to patient failure.

In another study of 102 cycles in 48 women, she used a sequential regimen of 11 days with 0.08 mg mestranol followed by 10 days with 2 mg chlormadinone+0.08 mg of mestranol. No pregnancy occurred during this regimen.

The cause for the higher pregnancy rate in our series may be that ethinyl oestradiol was used instead of mestranol and/or that the dosage of ethinyl oestradiol was too low The Birmingham oral contraceptive trial (Eckstein *et al.* 1962 Mears, 1961) indicates that the amount of oestrogen combined with 2 5 mg norethynodel, was critical and that a dose of 0.035 mg mestranol was insufficient.

In the early days of oral contraception it was assumed that different steroids blocked ovulation in the same way Today this concept has been modified. For example Overbeck and de Visser (1964) from experiments in rats, stated that 17 α-

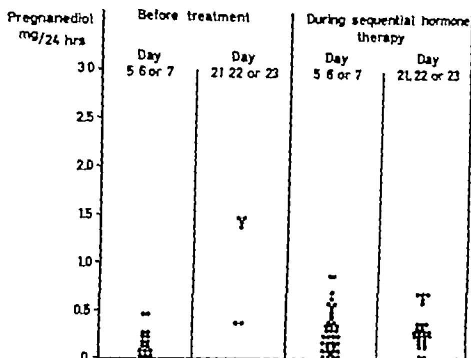


Fig 3 Urinary pregnanediol excretion before and during sequential hormone therapy (0 05 mg of ethinyl oestradiol for 20 days with 5 mg of medroxy progesterone acetate added during the last 10 days) The determinations were done on days 4 5 or 6 and repeated on days 20 21 or 22 of the cycle.

clusions can be drawn from the other differences registered, since Anovlar[R] was instituted after some months of sequential hormone therapy and since it is well known that the frequency of side effects decreases after the first month of oral contraception. Nine patients withdrew from the sequential therapy because of minor side effects

Six patients had daily estimations of LH excretion before and during treatment with sequential or combined therapy In all cases elevated pregnanediol values were found during the second half of the control cycle before treatment. In most women there was a rise in basal body temperature at mid-cycle.

The results of the assays of the daily urinary LH excretion during 25 cycles are shown in Figs 1 and 2

With the sequential therapy the urinary LH excretion was unchanged compared with the control cycles During the treat

SUMMARY

Two kinds of oral contraceptives were administered to 86 women during 437 cycles. The first preparation was administered as sequential therapy consisting of 0.05 mg of ethinyl oestradiol for 20 days with 5 mg of medroxyprogesterone acetate added for the last 10 days. Later the same patients were given a combination of 4 mg norethisterone acetate and 0.06 mg ethinyl oestradiol for 21 days. The effect of the two preparations were studied by determining the excretion of luteinizing hormone (LH) and pregnanediol.

Five pregnancies, 4 of which were due to tablet failure, occurred during the 210 cycles of sequential therapy the LH excretion was not significantly different from that of the control cycles. In contrast, Anovlar[R] caused a consistent decrease in the amount of LH excreted and no mid-cycle peaks were observed. The pregnanediol excretion was raised to a normal post-ovulatory level in 2 out of 28 cycles during the sequential therapy

Acknowledgement

The authors wish to thank dr Paul Roos, Institute of Biochemistry University of Uppsala, for the preparation of the human pituitary LH.

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ethinyl-estr 4-ene-17 β-ol (lynestrol) prevented ovulation by inhibiting FSH release from the pituitary In contrast, under the same experimental conditions 6-methyl-lynestrenol is shown to block ovulation by inhibiting the release of LH.

Our findings showed that in the two ways in which the steroids were used (sequential and combined therapy) they exerted a different effect upon the release of LH However this observation does not allow any conclusions to be reached as to how this mechanism operates

It is suggested that Anovlar[R] inhibits the secretion of LH and thus prevents ovulation. If "low pregnanediol values indicate that ovulation does not occur then the normal amounts of LH excreted by women on the sequential therapy suggest that inhibition occurs in a different way Furthermore, the use of steroids in some women may decrease the sensitivity of the ovary to gonadotrophic stimulation. Gemzell (1962) showed that progesterone (100 mg daily) prevented the effect of exogenous human pituitary FSH on ovarian size and oestrogen excretion, and Lunenfeld *et al* (1963) demonstrated that tablets containing 0 05 mg of ethinyl oestradiol and 5 mg of medroxyprogestrone acetate blocked the ovulation that otherwise could be induced by treatment with urinary gonadotrophins in patients with pituitary failure. Similar results with regard to the inhibition of gonadotrophin induced ovulation by tranquillizers progesterone and norethynodrol in immature mice were obtained by Purshottam *et al.* (1961) On the other hand, Edgren and Carter (1962) failed to demonstrate any block of HCG-induced ovulation in rabbits treated with various steroids

It has been known for a long time that the dose level is critical when oestrogens alone are used for blocking ovulation. Lipschütz (1950) concluded that, in the rat, moderate doses of oestrogens inhibit FSH activity and stimulate LH activity Others have shown that small doses of oestrogens stimulate the excretion of total gonadotrophins while larger doses decrease it (Diczfalusy and Lauritzen, 1961) The observations reported here support the concept that the mode of action of oral contraceptives is dependent upon the type of treatment, the kind of steroids the dose levels and the time schedule used.

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From the Hormone Laboratory Department of Obstetrics and Gynecology (Prof A. Ingelman-Sundberg) Sabbatsberg Hospital Karolinska Institutet Stockholm

OVARIAN STIMULATION BY HUMAN MENOPAUSAL GONADOTROPHIN IN PATIENTS WITH AMENORRHŒA AND ANOVULATION

BY

MIRJAM FURUHJELM, NILS OLOV LUNELL AND ERIK ODEBLAD

In recent years evidence has been reported indicating that gonadotrophic stimulation can produce ovulation in amenorrhoeic and anovulatory women who have absent or very low gonadotrophin excretion. Successful treatment with human pituitary gonadotrophin was first reported by Gemzell *et al.* (1958 1960 1962) Their results were confirmed by Bettendorf *et al.* (1961) and Buxton *et al.* (1963) Using gonadotrophins isolated from human menopausal urine similar results have been achieved by Lunenfeld *et al.* (1961) Rosemberg *et al.* (1963) Crooke *et al.* (1963) Diczfalusy *et al.* (1964) and Lunenfeld (1964)

Multiple ovulation, resulting in multiple pregnancies and the premature birth of up to 7 fetuses has occurred. The minimum dosage sufficient to induce maturation of only one Graafian follicle as in the normal menstrual cycle, is very difficult to estimate. Usually very high doses of gonadotrophins have been used. The purpose of our investigation is to study at reduced dosage levels, the ovarian function especially the steroid excretion pattern, the secondary effects on the cervical mucus and the possibility of obtaining maturation of one follicle and single pregnancies.

Although functional disturbances in the hypothalamic-pitui

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Table I. *Properties of the Different Batches of the Menopausal Gonadotrophins.*

Preparation	FSH-activity		FSH/LH ratio	
	IU/ml	IRPU/ml	IU/IU	IRPU/IRPU
HMG J 1.27	50	375	1.0	3.6
HMG J 1.4	100	750	1.4	4.8
HMG J 27.6	70	500	0.9	3.0
HMG J 27.14 B	70	500	9	3.0

mulating hormone (FSH). The FSH activity was assayed according to Steelman and Pohley (1953) and the luteinizing hormone (LH) activity according to Parlow (1958). Their FSH/LH ratios were relatively constant (Table I). The human chorionic gonadotrophin (HCG) preparation was a highly purified, commercially available product (Gonadex Leo (R) supplied by AB Leo Hälsingborg, Sweden).

Scheme of treatment. Doses of HMG varying between 100 and 1350 FSH units in the different cases were given intramuscularly for 2–10 days in 5 cases followed by injection of 6000 I.U. HCG for 4 days or 18000 I.U. in a single dose.

Assessment of clinical and steroid metabolic effects. The following parameters were examined: vaginal smear, cervical mucus, endometrium at biopsy, onset of uterine bleeding, ovaries at laparoscopy, urinary oestrogens, pregnanediol, 17-ketosteroids and 17-hydroxycorticosteroids.

Laboratory methods

The gonadotrophins were estimated as total urinary gonadotrophins during 24 hrs by observation of vaginal smears and the growth of follicles in infantile mice (Hamburger 1933). The pregnancy tests were performed according to Wide and Gemzell (1960).

Determination of steroids in urine

Œstrogens in µg/24 hrs (Furuhjelm and Waller 1958), pregnanediol in mg/24 hrs (Klopper et al. 1955), 17-keto-

tary-ovarian relationship are of varying aetiology the clinical picture is similar in all cases It is characterised by anovulatory bleeding, oligomenorrhoea or amenorrhoea with low excretion of gonadotrophins and pregnanediol and eventually of oestrogens also The disturbance can originate primarily in the ovary which may not be capable of reacting to stimulus from the pituitary or the ovary may have defective enzymatic systems leading to faulty steroid synthesis Trauma of the endometrium can also result in an inhibition of the oestrogenic effects as shown by de Jongh *et al* (1964) Cases of amenorrhoea might therefore develop after the endometrium has been traumatized by curettage or by infection.

The effect of the gonadotrophins given will thus depend not only upon the dose given, but also upon the disturbance which causes the actual disease. In our treatment we tried to adjust the dose of gonadotrophins according to individual circumstances.

Materials and Methods

The patients A total of 13 patients were included in this study None of them had an elevated gonadotrophin excretion. The diagnosis of a hypogonadotrophic amenorrhoea or anovulation was made on the basis of the case history physical examination, thyroid studies X-ray examination of the sella turcica, evaluation of the visual fields baseline steroid excretion levels basal temperature and visual examination of the ovaries at laparoscopy

The patients were divided into 3 groups

1) primary amenorrhoea	3 cases
2) secondary amenorrhoea,	6 „
3) anovulatory cycles	4 „

The relevant clinical data are summarized in Tables II, III and IV and completed by abstracts of the case histories given under the heading "Results of Treatment

The gonadotrophins. The preparations of human menopausal gonadotrophins (HMG) used were supplied by AB Leo Hälsingborg, Sweden, and derived from 4 different batches They were standardized against the second international reference preparation, their potency being expressed in units of follicle sti

Table I. *Properties of the Different Batches of the Menopausal Gonadotrophins.*

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HMG J 27 6	70	500	0.9	3.0
HMG J 27 14 B	70	500	0.9	3

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Determination of steroids in urine

Œstrogens in μg/24 hrs (Furuhjelm and Waller 1958) pregnanediol in mg/24 hrs (Klopper *et al.* 1955) 17-keto-

steroids in mg/24 hrs (Vestergaard, 1951) and 17 ketogenic steroids in mg/24 hrs (Birke *et al* 1958)

The vaginal smears

The hormonal activity was evaluated by the karyopyknotic index determined as the ratio of the karyopyknotic cells to all cells present, and expressed as a percentage.

The cervical mucus was evaluated by the amount, the arborization the fibrosity and the percentage of the dry substance.

Results of Treatment

The most relevant data are listed in Tables II–IV and additional comments are given in the abstracts of the case histories presented below

Group 1 Primary amenorrhoea (Table II)

a) *BJ* received in November 1964 daily injections of 70 FSH units (J 1 41) for 10 days followed by daily injections of 6000 LU of HCG for 4 days.

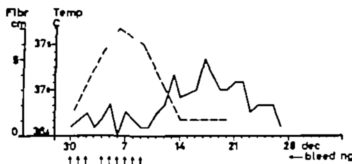


Fig. 1 Basal temperature and properties of cervical mucus in BJ with primary amenorrhoea treated with HMG 70 LU/day (↑) and HCG 6000 LU/day (↑)
Temperature ——— fibrosity ———

Table II *Clinical and Laboratory Data of Patients with Primary Amenorrhoea.*

Patient	Age	Secondary sex characters	Sex chromatin	Oestrogens	Vaginal cytology	Endometrium	Laparoscopy	Treatment	Result of treatment
B.I.	20	normal	pos	low	slight oestrogenic effect	atrophic	ovaries normal size a fresh corpus luteum	HMG 70 I.U./d < HCG 6000 I.U./d × 4	ovulation menstruation
I.J.	23	hypoplasia	pos	low	atrophic	atrophic	ovaries small no follicles	HMG 70 I.U./d × 7	slight response no bleeding
T.L.	22	hypoplasia	pos	low	atrophic	atrophic	small atrophic ovaries no follicles	HMG 70 I.U./d × 7	no response

Performed during treatment.

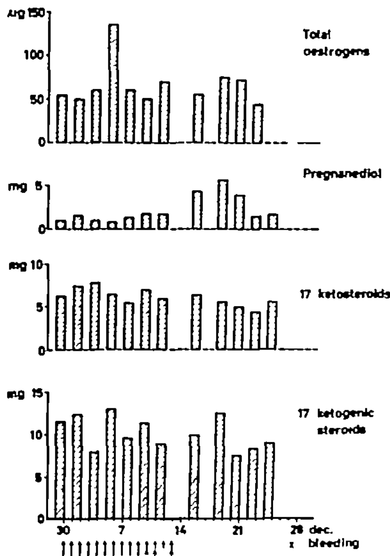


Fig 2. Excretion of steroids during treatment with HMG 70 I.U. per day (↑) and HCG 6000 I.U./day (↨) BJ primary amenorrhœa.

The cervical mucus became translucent abundant and showed arborization. There was later a rise in the basal temperature (Fig. 1) At laparoscopy performed 17 days after the start of the treatment one ovary contained 4–5 small follicles, and the other contained a fresh corpus luteum. Endometrial biopsy taken the same day revealed a secretory pattern. The urinary steroid pattern is seen in Fig 2. Menstruation started 24 days after beginning treatment. Two months later the patient had a normal spontaneous menstruation but since then has been amenorrhoeic.

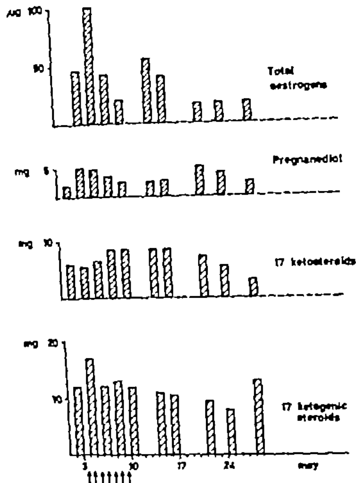


Fig 3. Excretion of steroids in I.J. during treatment with HMG 70 I.U./day(↑).

b) *I.J.* sister of the previous patient, *B.J.* was treated in March 1965 with a daily dose of 70 FSH units of HMG (*J 27-6*) for 7 days but this was not followed by HCG injections. She had a poor response to the treatment. The vaginal cytology revealed no oestrogenic influence. The cervical mucus increased, but was still scanty and without any arborization. There was no rise in the basal temperature, but there was a slight rise in the urinary excretion of oestrogens (Fig 3)

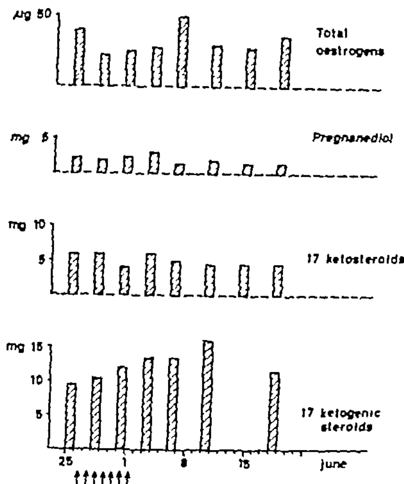


Fig. 4 Excretion of steroids in T.L. during treatment with HMG 70 I.U./day (↑)

c) T.L. also received daily injections of 70 FSH units for 7 days (J 27 14 B) *but did not respond. The vaginal epithelium remained atrophic, no secretion* of cervical mucus appeared and there was no rise of the urinary steroids except a slight possible increase of 17-ketogenic steroids (Fig 4)

Group 2. Secondary amenorrhoea (Table III)

a) *A.F.A.* had since the menarche, menstruated only 3–4 times a year When we started treatment in December 1963, she had been amenorrhoeic for a year She received 150 FSH units HMG (J 1 27) daily for 9 days followed by 4 injections of HCG 6000 I.U./day There was a moderate rise in the excretion of oestrogens, but no rise in the pregnanediol excretion. The ovaries became cystically enlarged up to the size of an orange, and 24 days after the

Tabl III. Clinical and Laboratory Data of Patients with Secondary Amenorrhea.

Patient	Age	Menarche	Amenorrhea years	U oestrogens	Vaginal cytology	Endometrium	Cervical mucus	Laparoscopy before treatment	Treatment	Result of treatment
A.F. A.	31	8		low	slight oestrogenic effect	not examined	scanty	not performed	HMG 70 LU/d < 0 HCG 6000 LU d× 4	withdrawal bleeding
M.J. E.	3	4	4	low	slight oestrogenic effect	not examined	scanty	not performed	HMG 50 LU/d× 4	pregnant
J.E.-H.	28		4	normal	medium oestrogenic effect	hypoplasia	scanty	ovaries enlarged many small follicles	HMG 70 LU/d× 4	no response
B.M. K.	24	4	3	low	atrophic	atrophic	scanty	not performed	HMG 70 IU/d×1 HCG 6000 LU/d× 4	pregnant
B.L.	22		0	low	atrophic	atrophic	scanty	ovaries normal size one follicl	HMG 70 LU/d×1 HCG 18000 LU/d×	pregnant
A.R.W.	20	2	5	normal	light oestrogenic effect	proliferative	scanty	ovaries enlarged no follicles	HMG 70 LU/d× 7	withdrawal bleeding

Performed during and treatment.

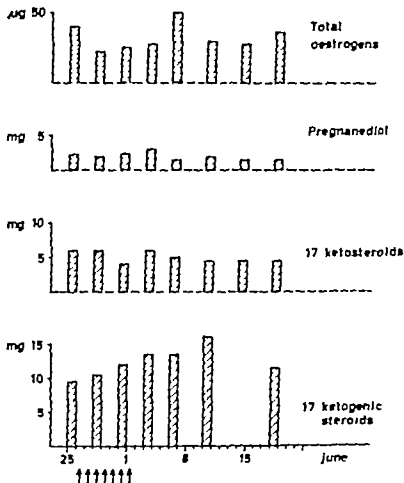


Fig. 4. Excretion of steroids in T.L. during treatment with HMG 70 I.U./day (↑)

c) *T.L.* also received daily injections of 70 FSH units for 7 days (J 27 14 B) but did not respond. The vaginal epithelium remained atrophic, no secretion of cervical mucus appeared, and there was no rise of the urinary steroids except a slight possible increase of 17-ketogenic steroids (Fig. 4).

Group 2. Secondary amenorrhoea (Table III)

a) *A.F.A.* had since the menarche, menstruated only 3–4 times a year. When we started treatment in December 1963, she had been amenorrhoeic for a year. She received 150 FSH units HMG (J 1. 27) daily for 9 days followed by 4 injections of HCG 6000 I.U./day. There was a moderate rise in the excretion of oestrogens, but no rise in the pregnanediol excretion. The ovaries became cystically enlarged up to the size of an orange and 24 days after the

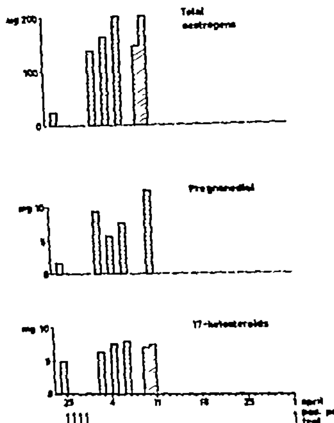


Fig. 8 Excretion of steroids in M. L.-E. during treatment with HMG 50 I.U./day (↑).

d) B.M.-K. (Table III) was treated with 5 injections of 00 FSH units of HMG (J 41) daily followed by HCG 6000 I.U. daily for 4 days. The karyopyknotic index of the vaginal epithelium rose from 6 per cent to 30 per cent. The cervical mucus showed arborization, and there was an increase in the excretion of oestrogens and pregnanediol. The basal temperature was elevated for 12 days suggesting ovulation and development of a corpus luteum. However she did not become pregnant, and on the 18th day bleeding occurred.

A new trial was performed 2 months later. This time the dosage was increased to 70 FSH units of HMG (J 27 6) per day for 10 days followed by

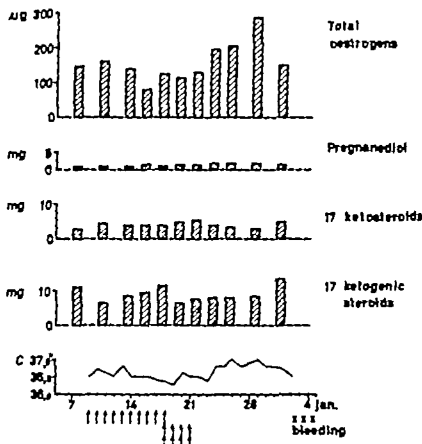


Fig. 5 Basal temperature and excretion of steroids in A.F A. during treatment with HMG 70 I.U./day (↑) and HCG 6000 I.U./day (↕)

treatment started withdrawal bleeding occured. In December 1964 she was treated again this time with 70 FSH units HMG (J 27 6) for 10 days followed by 6000 I.U HCG for 4 days (Fig. 5) As previously there was only a moderate increase in the oestrogen excretion, followed by withdrawal bleeding The ovaries were not enlarged.

b) *M.J E.* (Table III) was treated only with 50 FSH units of HMG (J 1 27) for 4 days (Fig. 6) There was an obvious rise in the excretion of oestrogens and pregnanediol. Five weeks later a pregnancy test was positive. She delivered, at term, a normal healthy boy weighing 3400 g.

c) *J.E.-H* (Table III) received 4 injections of 70 FSH units of HMG (J 27 6) without any response. At laparatomy 2 months later the diagnosis of Stein-Leventhal syndrome was proved.

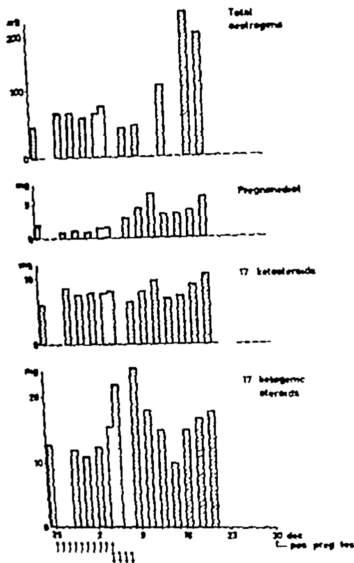


Fig 8 Excretion of steroids in B M -K. treated with HMG 70 I U per day ( ) and HCG 6000 I U/day (↕)



Fig. 7 Basal temperature and properties of cervical mucus in B.M.-K. treated with HMG 70 I U./day (↑) and HCG 6000 I.U./day (↑) Temperature —— fibrosity ——

HCG 6000 I.U. daily for 4 days. The changes in basal temperature and cervical mucus are shown in Fig 7 and the excretion of urinary steroids in Fig 8 It is likely that ovulation occurred on the first or second day after the injections of HCG were started She became pregnant and delivered at term a healthy child, weighing 3670 g

e) *B.I* (Table III) menstruated twice at the age of 12. Following a severe attack of rubella secondary amenorrhoea developed, and there was poor development of her secondary sex characters. Before receiving treatment with gonadotrophins cyclic oral treatment with oestrogens and progestagens for 3 months showed an adequate response of the cervical glands and the endometrium. In November 1964 she was given 70 FSH units of HMG (J 27-6) daily for 10 days. There was a pronounced oestrogenic response of the cervical mucus (Fig. 9) The urinary excretion of oestrogens rose to high values and the excretion of pregnanediol showed a slight rise (*Fig. 10*) Withdrawal bleeding followed after 15 days.

In a second trial in January 1965 she received the same dose of HMG followed by injection of 6000 I.U. of HCG daily for 4 days At laparoscopy on the ninth day of treatment a mature follicle was found in one ovary The basal temperature increased to 37.2 C for 12 days. The excretion of pregnanediol rose to 3.9 mg/24 hrs one week after the first injection of HCG The patient did not deliver urine samples after this day

The patient was treated again in May 1965 She received 70 FSH units of HMG daily for 10 days. Simultaneously with the last injection of HMG she was given one single injection of 18000 I.U. of HCG This time she became pregnant (Fig. 11)

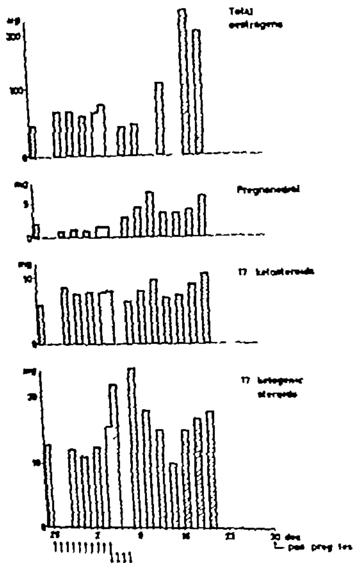


Fig. 8 Excretion of steroids in B.M.-K. treated with HMG 70 I.U. per day (↑) and HCG 6000 I.U./day ( ).

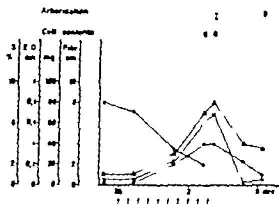


Fig. 9 Properties of cervical mucus in B.I. during treatment with HMG 70 I.U./day (↑) D.S = per cent dry substance Am = amount and Fibr = fibrosity E.O = diameter of external os.

μg 200
100
Total oestrogens

mg 5
Pregnanediol

mg 10
5
17 ketosteroids

mg 15
10
5
17-ketogenic steroids

25 2 9 16 dec
---- slight bleeding

Fig. 10. Excretion of steroids in B.I. during treatment with HMG 70 I.U./day (↑)

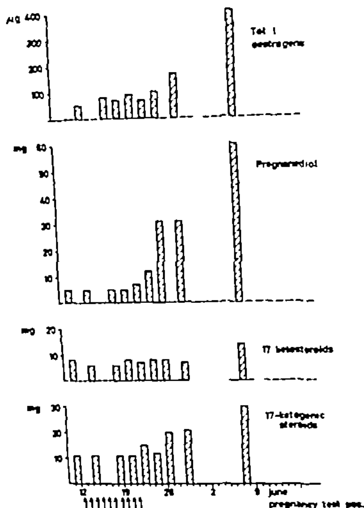


Fig. Excretion of steroids during 3rd treatment of B.L. with HMG 70 I.U./day (↑) and HCG 8000 I.U. ( )

f) A.R.W. (Table III) menstruated regularly until 5 years before treatment, when she had traffic accident with cerebral concussion. Before treatment she had normal excretion of oestrogens in the urine. When treated with 70 FSH units of HMG (J 27-6) daily for 7 days, she responded with highly increased urinary levels of oestrogens. The cervical mucus increased and

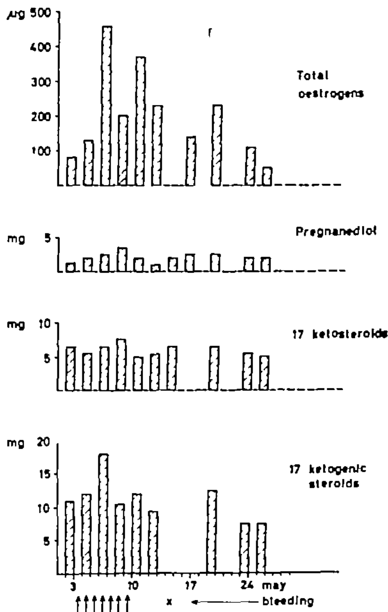


Fig. 12. Excretion of steroids in A.R.W. during treatment with HMG 70 I.U./day (↑)

showed arborization. One ovary became enlarged. Five days after the last injection withdrawal bleeding occurred. (Fig. 12)

3. *Patients with anovulatory cycles*

Four patients with anovulatory cycles have been treated with small doses of HMG

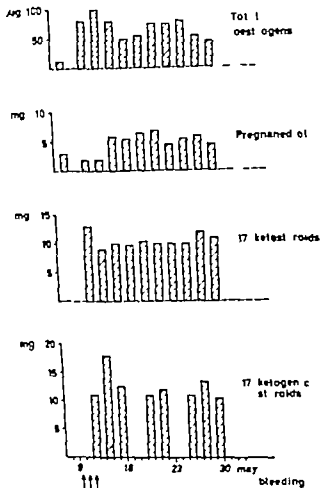


Fig 3 Excretion of steroids in B.J. (Table IV) treated with HMG 70 I.U./day (↑)

) B.J (Table IV) received 70 FSH units of HMG (J 27 6) daily for 3 days. She responded with urinary excretion of pregnanediol up to secretory levels for 4 days, whereupon menstruation followed (Fig 3)

b) G.M (Table IV) had previously been treated for cystic glandular hyper plasia of the endometrium with injections of progesterone. During the fol-

Table IV *Clinical and Laboratory Data of Patients with Anovulatory Cycles.*

Patient	Age	Menarche	Menstruation	Parity	Oestrogens γ/24 hrs	Pregnanediol mg/24 hrs	Oestrogenic mucus	Treatment (I U /day)	Result of treatment E—oestrogen P—pregnanediol
B.J.	38	14	regular 5/28 d	0	22	2.2	+	HMG 70×3	rise of E rise of P to secretory values
G.M.	30	12	treated for endometrial hyper plasia, periods now regular 5/28 d	0	—	1.7	+	HMG 70×4 Febr	rise of E rise of P to secretory values
					52	1.6	+	HMG 70×3 April	rise of E no rise of P
					44	2.1	+	HMG 70×4 June	rise of E rise of P to secretory values
S.L.	28	13	irreg. 5/28–35 d		23	2.2	+	HMG 70×3 March	rise of E no rise of P
				0	41	2.2	+	HMG 70×9 HCG 6000×4 May	no effect
M.I.	26	14	irreg. 3–5 mths interv	1	26	1	+	HMG 00×3	ovulated and menstruated twice then pregnant

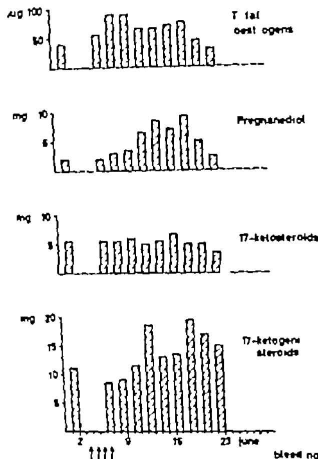


Fig 4 Excretion of steroids in G.M. treated with HMG 70 I.U. per day (↑).

lowing 3 years she menstruated at regular intervals but the cycles were anovulatory In February 965 she was treated with 70 FSH units of HMG (J 27 6) for 4 days The basal temperature rose to 37 and remained at this level for 4 days until the patient menstruated. The urinary output of oestrogens rose to very high levels and the urinary excretion of pregnanediol reached secretory phase levels and remained high until the menstruation. The patient was treated again in April 1965, this time with injection of 70 FSH units of HMG (J 27 6) daily for only 3 days. No rise in the pregnanediol excretion appeared The dose given was thus too low In June 1965 third

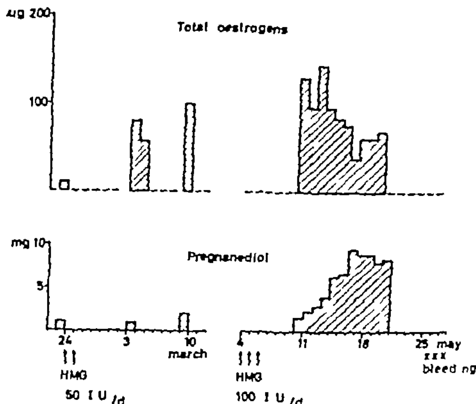


Fig 15. Excretions of oestrogens and pregnanediol in M.L. treated with two different doses of HMG

treatment was started, with 70 FSH units of HMG (J 27·14 B) daily for 4 days, as was given the first time. She ovulated (Fig. 14) but did not become pregnant. Therefore we examined the semen of her husband. The count showed 69.5 per cent abnormal spermatozoa. This fact was probably the ex planation of the failure.

c) S.L. (Table IV) received 70 FSH units of HMG (J 27·6) daily for 3 days. She responded with a moderate rise in the excretion of oestrogens in the urine, but had no rise in the excretion of pregnanediol.

Next, she was treated with 70 FSH units of HMG (J 27·6) daily for 9 days, followed by injection of 6000 I.U. HCG for 4 days. In spite of the intensified treatment she did not respond.

d) M.L. (Table IV) always had irregular menstrual cycles with long periods of amenorrhoea. In 1962 she was treated with pregnant mare serum gonadotrophin (PMS) and HCG and became pregnant. After a normal delivery in June 1963 she had no ovulatory cycles but on three occasions had with-

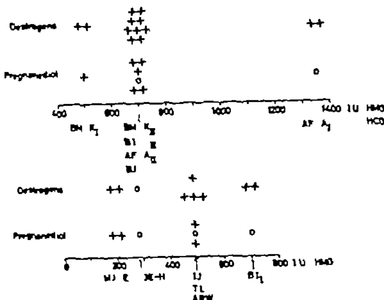


Fig. 16 The oestrogen and pregnanediol response of the amenorrhoeic patients to different doses of HMG with HCG (top) and without HCG (bottom) The urinary steroid response is graded + ++ and +++ corresponding to no, low moderate or high excess.

drawal bleeding. In February 1964 she was treated with injections of 90 FSH units of HMG (J 37) and responded with a rise in the oestrogen excretion and bleeding 4 weeks after the injections. In May 1964 she was treated with 00 FSH units of HMG (J 14) daily for 3 days, and responded with formation of a corpus luteum as judged by the increase of basal temperature and the level of pregnanediol in the urine (Fig. 5). Menstruation followed and occurred again 4 weeks later On the first of August (the following cycle) there was rise in the basal temperature and a pregnancy test was positive on August 24th. She delivered healthy boy weighing 4150 g on April 22nd, 965

The response of the patients to the different HMG-doses with or without HCG is schematized in Fig. 16.

Discussion

Our results show that HMG with or without consecutive treatment with HCG can induce ovulation and formation of corpus

luteum in certain amenorrhoeic women and in women with anovulatory cycles The ova are capable of being fertilized as verified by the resulting pregnancies.

The result of the treatment does not only depend upon the dosage given (Fig 16) but some other factor must be of crucial importance. This factor presumably resides in the ovary and may be the state of the follicular system at the time of HMG administration. In certain cases the ovary is not capable of responding to gonadotrophic stimulation although the gonadotrophic excretion in the urine before treatment is not increased. This is the case in one of our patients (T.L.) with primary amenorrhoea, who had small atrophic ovaries If the cause of the amenorrhoea is a too low or an altered hypophyseal release of gonadotrophin, exogenously administered gonadotrophins ought to stimulate the ovaries and induce ovulation. This has also happened in our patients with insufficient stimulation from the pituitary They did respond with ovulation and pregnancy after administration of an apparently sufficient dose of HMG followed by HCG The problem in these cases however is to avoid a too high dosage followed by multiple pregnancies It is therefore wise to start with a low dosage even if it is insufficient to cause ovulation the first time. Nevertheless such treatment will stimulate the ovaries and probably increase their sensitivity to gonadotrophic stimulation, which may be of value especially in cases with longstanding amenorrhoea. Then when moderate doses of HMG followed by HCG are used ovulation can occur as demonstrated in case B.L (Figs 10 and 11)

In our cases of secondary amenorrhoea a total dose of 700 FSH units followed by HCG either as 6000 I.U for 4 days or as one single injection of 18000 I U HCG was capable of inducing ovulation and pregnancy In one case (M.J) however only 4 injections of HMG totalling 200 FSH units resulted in pregnancy and normal delivery The occurrence of ovulation and formation of a corpus luteum following HMG injection only may be explained by an endogenous release of LH secondary to an increased oestrogen production following the HMG stimulation.

Patients with anovulatory cycles may have only a slight disturbance in the hypothalamic-pituitary-ovarian relationship As

suming that their ovaries do not produce enough oestrogens to cause adequate LH release, an adequate treatment should be stimulation of the ovaries with small doses of HMG in order to increase their oestrogen production to a level sufficient for the release of LH. Therefore our four patients with anovulatory cycles were treated with a total dose of only 100–300 FSH units of HMG without any additional HCG. Three of them responded with excretion of pregnanediol to secretory levels. One of them (S.L.) was treated in a second trial with 70 FSH units of HMG daily for 9 days, followed by 6000 I.U. HCG daily for 4 days. This time she showed no response.

Land (1965) has pointed out that there must be an interaction between the exogenous hormone administrated by injection, and the endogenous follicle stimulating system. If there is a sufficient release of endogenous gonadotrophins from the pituitary the exogeneous hormone, if given in high doses might suppress the pituitary without stimulating the ovary. Gemzell (1958) has also reported a similar case, who had a normal excretion of oestrogens and gonadotrophins, but who did not respond to treatment with high doses of HMG followed by HCG.

By the low dosage of HMG used by us it has also been possible to obtain an excretion pattern of urinary oestrogens, resembling that of a normal cycle, as seen in Figs. 2 and 13.

SUMMARY

Three patients with primary amenorrhoea, 6 with secondary amenorrhoea and 4 with anovulatory cycles have been treated with human menopausal gonadotrophin (HMG). In 5 patients injections of human chorionic gonadotrophin (HCG) followed. The clinical and steroid effects were evaluated by vaginal smears, endometrial biopsies properties of cervical mucus, inspection of ovaries at laparoscopy and determinations of the 24 hrs urinary excretion of oestrogens, pregnanediol, 17-ketosteroids and 17-hydroxycorticosteroids.

The doses [illegible] used for the 3 patients with primary amenorrhoea var[illegible] FSH units of HMG. One patient [illegible] showed signs of

luteum in certain amenorrhoeic women and in women with anovulatory cycles The ova are capable of being fertilized as verified by the resulting pregnancies

The result of the treatment does not only depend upon the dosage given (Fig 16) but some other factor must be of crucial importance. This factor presumably resides in the ovary and may be the state of the follicular system at the time of HMG administration. In certain cases the ovary is not capable of responding to gonadotrophic stimulation although the gonadotrophic excretion in the urine before treatment is not increased. This is the case in one of our patients (T.L.) with primary amenorrhoea, who had small, atrophic ovaries If the cause of the amenorrhoea is a too low or an altered hypophyseal release of gonadotrophin, exogenously administered gonadotrophins ought to stimulate the ovaries and induce ovulation. This has also happened in our patients with insufficient stimulation from the pituitary They did respond with ovulation and pregnancy after administration of an apparently sufficient dose of HMG followed by HCG The problem in these cases however is to avoid a too high dosage, followed by multiple pregnancies It is therefore wise to start with a low dosage, even if it is insufficient to cause ovulation the first time. Nevertheless such treatment will stimulate the ovaries and probably increase their sensitivity to gonadotrophic stimulation, which may be of value especially in cases with longstanding amenorrhoea. Then when moderate doses of HMG followed by HCG are used ovulation can occur as demonstrated in case B.I. (Figs 10 and 11)

In our cases of secondary amenorrhoea a total dose of 700 FSH units followed by HCG either as 6000 I.U for 4 days or as one single injection of 18000 I.U HCG was capable of inducing ovulation and pregnancy In one case (M.J) however only 4 injections of HMG totalling 200 FSH units resulted in pregnancy and normal delivery The occurrence of ovulation and formation of a corpus luteum following HMG injection only may be explained by an endogenous release of LH secondary to an increased oestrogen production following the HMG stimulation.

Patients with anovulatory cycles may have only a slight disturbance in the hypothalamic-pituitary-ovarian relationship As

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increased oestrogen production, and the third one, with small atrophic ovaries, did not respond.

The dosage of HMG used for the 6 patients with secondary amenorrhoea varied between 200 and 700 FSH units One patient showed no response Three patients became pregnant, and one of them two had also received HCG

Four patients with anovulatory cycles were treated with 210-300 FSH units of HMG The doses given were sufficient to induce urinary excretion of pregnanediol to secretory phase levels and to cause a rise in the basal temperature.

Signs of an increased oestrogen production appeared in all patients except one with low excretion before treatment. A cor *relation between oestrogen and pregnanediol excretions and the* dose of HMG in the amenorrhoeic groups was not indicated.

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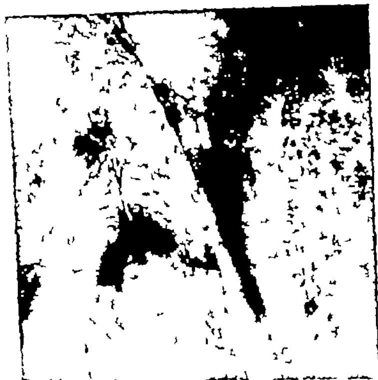


Fig.

Material

This comprises 42 cases from the Norwegian Radium Hospital which have been photographed with construction of composite pictures. Fifteen cases were normal or had benign lesions, three were precancerous lesions, one an early invasive lesion and 20 cases were primary vulvar carcinoma (including 3 cases of urethral carcinoma). In addition there are 3 metastatic vulvar carcinomas. All colpophotographs in this paper are given at a scale of 10 : 1.

Normal findings are relatively simple to describe as compared with those of the uterine cervix. On the labia majora the capillaries are hardly visualized without previous application of oil

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COLPOPHOTOGRAPHY AS AN AID IN THE STUDY OF VULVAR LESIONS

BY

ODDMUND KOLLER

Introduction

It is generally realized that the study of the vulva is relatively neglected although the vulva is the site of a great many lesions of obscure clinical significance. One of the reasons for this may be that naked eye inspection often gives very little clue as to the true nature of a lesion, and the sensitivity of the area does not allow biopsy as easily as for instance, the cervix. Vulvar lesions often involve large areas and the site to be chosen for a biopsy in order to get the most representative specimen, is not easily determined.

It has been suggested that the colposcope could be used as an aid in the examination of a large variety of vulvar lesions in forensic medicine to establish the state of the hymen (Wespi, 1948 Beric 1956) in kraurosis and inflammatory lesions (Mestwerdt and Wespi, 1961) in the diagnosis of urethral polyps (Mestwerdt, 1953) and of vulvar carcinoma (Ganse, 1953 Kirsch and Rieck, 1954)

Since 1956 a special colpophotographic technique has been applied at the Norwegian Radium Hospital for the demonstration of lesions of the uterine cervix (Koller 1963 Koller and Kolstad, 1963 Bergsjø Koller and Kolstad, 1963 Kolstad, 1964) and it was natural to use this technique for the study of vulvar lesions as well. In this paper some of the results of the study are presented.

Fig. 3.





Fig 2

to the skin. On the labia minora and especially on their medial and moist part, as in the vestibule area including the urethral orifice, the vessels are more readily visible. The capillaries are of the hair-pin type about 0 1 mm. apart and frequently lineally arranged forming a finger print like pattern. Surrounding the urethral orifice and also the Skenes ducts the capillary rows are frequently arranged in a mosaic like fashion.

Fig. 1 illustrates the external urethral orifice and the medial part of the left labium minor

Fig 2 shows a small and Fig 3 a larger urethral polyp Note that the mosaic vascular patterns have been preserved, even though the fields are larger and the vessels more coarse than in the normal picture

Fig 4 shows a histological section of Fig 3 lesion and demonstrates the broad epithelial pegs separated by slender stromal clefts containing the vessels.



Fig. 7

Fig. 5 is a colpophotograph of condylomata acuminata located in the urethral orifice not unlike a cluster of raspberries. The papillary excrescences on the surface are mostly of the whitish, rather opaque appearance, but in several of them a centrally located, enlarged, hairpin-like capillary is clearly visualized or suggested. A histological section of the lesion is shown in Fig. 6 In contrast with Fig. 4 the broad epithelial layers ha e a papil

Fig. 5.



Fig. 6.



Fig. 9.

lomatous arrangement with a centrally located stromal core containing large capillaries, which is exactly the tissue-architecture to be expected from the colpophotographic picture.

The 3 cases with histological findings suggesting precancerous lesions were all well demarcated with leucoplakic areas of mosaic appearance. Where the vessels were visible the crests were somewhat enlarged and the tone of the tissue was darker than that of the surrounding area. Such findings are illustrated in the periphery (on the right side) in Fig. 7. More centrally (upper left) the capillaries are still larger and the surface is definitely raised and irregular with formation of flat nodules. Histologically



Fig. 8



Fig.

irregular in shape and arrangement. In three quarters of the cases there were branching vessels as seen in Fig. 10 and Fig 11 Characteristically in all cases of invasive squamous carcinoma there was increased capillary distance with irregular a vascularized areas, which in some cases could be very large

There seems to be a fairly constant correlation between vascular pattern and tissue architecture as revealed in the histological sections. In precancerous lesions and early invasi e lesions the enlarged capillary loops seen at colposcopy correspond to the elongated connective tissue papillae demonstrated in the histological sections (See Fig 12) In frankly invasi e lesions (Fig 13) there are larger and smaller irregularly arranged compact



Fig. 10.

the peripheral part showed a picture with atypical hyperplasia and elongated connective tissue papillae, consistent with that of a precancerous lesion. Centrally there was early stromal invasion.

Relatively large areas with colposcopical and histological findings consistent with precancerous lesions could also be seen in the periphery of frankly invasive lesions. Fig. 8 shows a part of the precancerous lesion and Fig. 9 a part of the invasive squamous cell carcinoma of the right labium minor.

All invasive lesions were characterized by pronounced changes of the surface contour (exophytic or endophytic) and markedly abnormal vascular patterns. The capillaries were enlarged and



Fig. 1

irregular in shape and arrangement. In three quarters of the cases there were branching vessels as seen in Fig. 10 and Fig. 11. Characteristically in all cases of invasive squamous carcinoma there was increased capillary distance with irregular avascularized areas, which in some cases could be very large.

There seems to be a fairly constant correlation between vascular pattern and tissue architecture as revealed in the histological sections. In precancerous lesions and early invasive lesions the enlarged capillary loops seen at colposcopy correspond to the elongated connective tissue papillae demonstrated in the histological sections. (See Fig. 12.) In frankly invasive lesions (Fig. 13) there are larger and smaller irregularly arranged compact

An. 7* 63

Fig 1

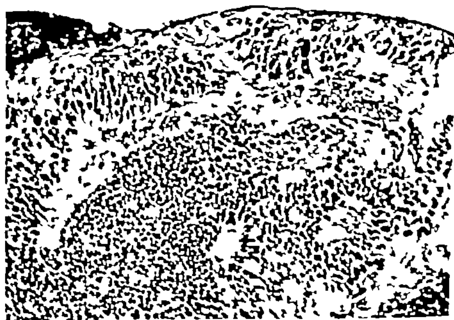


Fig 13



Fig. 14

masses of tumour cells an architecture which fits well with the colpophotographic appearance characterized by an atypical, often branching vascularization.

The metastatic tumours were two adenocarcinomas and one squamous cell carcinoma, the latter being shown in Fig. 14. The patient had had a cervical carcinoma with a similar vascular pattern, hairpin-shaped capillaries partly centrally located in papillary projections. The most impressive difference between this picture and Fig. 5 is that in Fig. 14 the vessels are more readily visualized, which means that the atypical epithelium is more translucent than the normal epithelium.

Fig 12

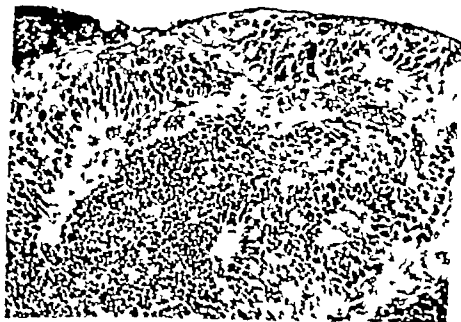


Fig 13.

It is concluded that colposcopy and colpophotography of vulvar lesions may be useful both clinically and in research.

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Discussion

The present study supports the claim by previous authors that the colposcopical examination method may provide information of clinical significance in vulvar lesions Especially by analysing the vascular pattern it is possible to obtain an idea of the tissue architecture of the pathological lesion The vascular pattern can easily be studied with an ordinary colposcopical technique in the vestibulum and on the medial parts of the labia minora but not in the areas covered with skin. It is possible that the latter areas could be studied statisfactorily after application of oil. Gilje (1953) has proved that capillary microscopic examination may be employed to advantage in dermatology both in differential diagnosis and general research The colpophotographic findings presented were much of the same type as those found on the uterine cervix. The normal findings were characterized by a dense and uniform distribution of fine capillaries The precancerous lesions and cancer were sharply delineated and deviated from the normal picture as to the tone, surface contour and vascular pattern. The characteristic trend of the vascular pattern was the reduced capillary density A branching type of abnormal vascularization was tantamount to an invasive lesion (Koller 1963 Kolstad, 1964)

Experience with colposcopy in vulvar lesions so far indicates that the method may be useful in practical clinical work Perhaps more important may be the fact that colposcopy and colpophotography make it easier to study vulvar lesions in detail and follow their development, progression or regression, than would be possible solely by the combination of naked eye inspection and biopsy for histological examination.

SUMMARY

A series is presented of 42 patients with vulvar lesions examined with a colpophotographic technique especially designed for visualizing the vascular pattern.

The colpophotographic findings were much of the same type as those found on the uterine cervix when examined by a similar technique.

ect of Octapressin on bleeding from parenchymatous tissue compared on a molar basis, is almost ten time as strong as that of epinephrine (Berde, 1961) An excellent review of the pharmacology of vasopressin has been published by Berde (1963)

Infiltration with epinephrine can cause local cyanosis and tissue hypoxia resulting in tissue damage with an increased tendency to postoperative oedema and bleeding and consequent impairment of healing. Octapressin appears to have a less toxic local effect on the tissues. When added to a local anaesthetic (5 I.U. Octapressin per 30 ml of a local anaesthetic solution) it prolongs the anaesthesia to the same extent as epinephrine (Klingenström and Westermark, 1963)

Material and Methods

In those patients about to undergo conisation because of cytologically suspect cancer of the uterine cervix epinephrine and Octapressin were used alternately as vasoconstrictors. All of the 37 patients were treated in hospital and all of them were healthy except for the condition for which they were to be operated upon. The patients ages ranged between 17 and 54 years.

The mixture used consisted of 30 ml of freshly prepared solution of 1 ml of adrenalin 1 100 000 or Octapressin (5 I.U.) per 100 ml physiological saline. The operation was otherwise performed according to a standard technique with deep sutures placed on the cervical uterine branches on either side before infiltration and *excision of the cone. All operations* were performed under general anaesthesia as a standard routine.

The patients were examined with respect to the following *factors.*

1 Immediate haemostatic effect. This effect was appraised by the surgeon and one (E.W.) of us who attended all operations. The effect was graded as good or moderate

2. Post-operative haemorrhage. Such bleeding was controlled by re-infiltration with the same haemostatic as that used at the operation.

3. Electrocardiographic changes after infiltration. Electrocar

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A SYNTHETIC VASOPRESSIN (OCTAPRESSIN SANDOZ) FOR HÆMOSTASIS DURING CONE BIOPSY OF THE CERVIX

BY

STIG KULLANDER AND ERIKA WIDE

In the surgical technique for cold knife conisation of the portio described by Scott, Welch and Blake (1960) the cervix is infiltrated with a vasoconstrictor to reduce blood loss and the wound is left unsutured. Satisfactory infiltration of the portio results not only in vasoconstriction but also in ballooning of the portio This facilitates the placing of the incision well outside the affected or suspect area Formerly we used epinephrine solution (2–3 drops of epinephrine 1 1 000 per 10 ml of physiological saline) for infiltration in accordance with the recommendations of Scott, Welch and Blake. In a clinical trial starting at the end of 1962 we tried synthetic phenylalanine lysine vasopressin (Octapressin, Sandoz) a vasopressin analogue which is said not to cause the occasionally disconcerting, sudden increase in blood pressure and increase in the pulse rate known to attend the use of adrenalin but nevertheless having the desired local vascular effect

Another advantage should be the compatibility with modern inhalation anaesthetics particularly with Halothane (Shanks, 1963 1964)

In contrast to natural vasopressin, the synthetic analogue is a potent vasoconstrictor with little or no oxytoxic or antidiuretic effect. Thus, in rats its depressive effect on the excretion of urine is only one third of that of Vasopressin. The ischaemic ef

Reinjection of Octapressin (3 cases) or epinephrine (4 cases) had an equally good effect on such later bleeding. In one of the patients in the Octapressin group suturing to stop the bleeding was used instead of a second injection of the vasoconstrictor

V. H. 3097/63, 22 year old Para I. The patient had 6 years previously had rheumatic fever and had since had cardiac symptoms and intermittent E.C.G abnormalities inverted P waves as in nodal rhythm. Two years before operation she had undergone laparotomy because of the Stein-Leventhal syndrome and had afterwards become pregnant. During pregnancy she was depressed and had suicidal thoughts and it was then that cytological smears had shown signs of carcinoma, for which she was subjected to conisation after normal delivery. One hour after conisation she developed a state of excitation, and she did not emerge from the narcosis until after further hour. Diffuse bleeding from the wound cavity was observed 2–3 hours after the operation. She was not retreated with Octapressin because of the possibility that the excitation might have been due to the Octapressin given at the operation. Electroencephalography the day after the operation showed moderate diffuse abnormality which regressed after week. After the patient regained consciousness she felt well and healing was uncomplicated.

The incidence of bleeding in the relation to the histological appearance of the operative specimens showed that primary bleeding or late bleeding (a total of 11 cases) occurred only in patients in whom cancer *in situ* was histologically confirmed (27 cases). The present series is not large enough to decide whether bleeding is less common in patients with a benign histological picture of the cervix (10 cases in this series) than in those with carcinoma *in situ*.

The effect of Octapressin and epinephrine on the circulation was appraised from the following four electrocardiographic features: cardiac rate, appearance of ST interval and T waves and rhythm.

1. *Cardiac rate*

	Octapressin	Epinephrine
Unchanged	1	3
Increased (>5 beats per min.)	2	3
Decreased (5–20 beats per min.)	7	9
„ (>20 beats per min.)	8	4

diograms were recorded after the anæsthesia had become stable and then again 3 minutes and 8 minutes after infiltration with epinephrine or Octapressin. Three leads were used (left arm-right arm, right arm left leg, and left arm-left leg)

4 Changes in blood pressure. During electrocardiography the blood pressure was measured with a Riva Rocci cuff with the arm at the level of the heart.

5 Changes in the secretion of urine during the first 6 hours after the operation This was appraised in some patients from the amounts of urine collected with an indwelling catheter and measured hourly

The effect of the preparations were also studied for any variation with the patients ages, parity and the histopathological appearance of the operative specimens

Results

The vasoconstrictor effect of Octapressin in the operative field was at least as good as that of epinephrine.

Table I. *Immediate hæmostasis*

Octapressin		Adrenaline	
Good	17 cases	Good	17 cases
Moderate	1 case	Moderate	2 cases

Radical extirpation of a histologically confirmed carcinoma *in situ* in a 22 year old woman who had born 1 child and in whom a second pregnancy had terminated in abortion.

Radical extirpation of a histologically confirmed carcinoma *in situ* in a 35 year old woman who had born 1 child and in whom a second pregnancy had terminated in abortion.

Radical extirpation of a histologically strongly suspected carcinoma *in situ* in a 27 year old woman who had born 3 children.

Later bleeding occurred equally often after both vasoconstrictors.

Table II. *Later Bleeding*

Octapressin	Adrenaline
4 cases	4 cases

Conduction disturbances were seen in both the epinephrine and the Octapressin groups. The present investigation would not allow appraisal of the effect, if any of the anesthesia.

In the analysis of the blood pressure changes "increased" is to be understood as an increase of the systolic blood pressure by at least 30 mm Hg (with no proportional change in the pulse amplitude) compared with the original value.

Table III. *Systolic Blood Pressure*

	Octapressin	Adrenaline
Increased	3	8
Unchanged	5	13

It is clear from Table III that epinephrine more often tended to raise the systolic blood pressure.

In many of the patients treated with Octapressin decreased pulse amplitude, but no increase in the systolic blood pressure occurred. In 9 of the patients in the Octapressin group an increase was noted mainly or only in the diastolic blood pressure, but in none of the patients in the epinephrine group. Such a diastolic increase together with the decreased cardiac rate after Octapressin injection, probably results in a decreased cardiac out put (also observed in dog experiments by Hamlin, 1963)

The frequently marked and occasionally prolonged pallor of the skin in patients treated with Octapressin is of no clinical importance and is due to the vasoconstrictor effect of Octapressin differing qualitatively from that of epinephrine. In the terminal vascular bed epinephrine acts upon the capacitance and resistance vessels, while Octapressin *probably acts* mainly on the capacitance vessels and the postcapillary venules (Berde and Cerletti, 1964)

Octapressin inhibited the secretion of urine more than adrenalin.

The inhibiting effect of Octapressin on the secretion of urine was conspicuous but did not interfere with the patient s well-being. The effect lasted for several hours.

The tendency of cardiac rate to decrease was thus more marked among the patients treated with Octapressin. A moderate in crease of the systolic blood pressure and bradycardia have been reported after intravenous injection of Octapressin (Guhl, 1961 Hugin 1962)

2 *ST* interval

	Octapressin	Epinephrine
Unchanged	17	15
Decreased (1–2 mm)	0	4

An undesirable effect, a sign of discrepancy between oxygen demand and oxygen supply of the myocardium was noted in 4 of the patients treated with epinephrine but in none of those treated with Octapressin Owing to unsatisfactory technique the ST interval could not be analyzed in one of the cases treated with Octapressin.

3 *T-waves*

	Octapressin	Epinephrine
Unchanged	16	8
Flattening	1	7
Diphasic	1	4

Epinephrine certainly had a more unfavourable effect on the myocardium than Octapressin ($0.001 < P < 0.01$) In patients with a history of myocardial injury or coronary insufficiency infiltration with epinephrine may imply a risk E.C G changes have not been observed even after intravenous injection of Octapressin (3 I V) (Guhl 1961 Hugin cit from Hochuli, 1962)

4 *Rhythm*

	Octapressin	Epinephrine
Unchanged	16	18
Arrhythmia (supraventricular or ventricular extrasystole)	2	1

Conduction disturbances were seen in both the epinephrine and the Octapressin groups. The present investigation would not allow appraisal of the effect, if any of the anesthesia.

In the analysis of the blood pressure changes "increased" is to be understood as an increase of the systolic blood pressure by at least 30 mm Hg (with no proportional change in the pulse amplitude) compared with the original value.

Table III. Systolic Blood Pressure

	Octapressin	Adrenaline
Increased	3	8
Unchanged	15	3

It is clear from Table III that epinephrine more often tended to raise the systolic blood pressure.

In many of the patients treated with Octapressin decreased pulse amplitude, but no increase in the systolic blood pressure occurred. In 9 of the patients in the Octapressin group an increase was noted mainly or only in the diastolic blood pressure but in none of the patients in the epinephrine group. Such a *diastolic increase* together with the decreased cardiac rate after Octapressin injection, probably results in a decreased cardiac output (*also observed in dog experiments* by Hamlin, 1963)

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Table IV *Urine Output*

	Number of Patients Studied After Use Of Octapressin 8	Adrenaline 6
<30 ml/hr during first 3 hours after operation	5	2
<30 ml/hr 3–6 hours after operation	6	2
Total <100 ml during first 6 hours after operation	6	1

The effects of Octapressin and epinephrine were also studied for any variation with the patients ages and parity The distribution in both groups was approximately the same in these two respects and no variation was found with age or parity

Discussion

Conisation of the portio not infiltrated with a suitable vasoconstrictor may be associated with considerable blood loss making the actual incision more difficult. But in a properly infiltrated cervix the portio is pale or even white and incision is followed by minimal bleeding from the wound surface. As a rule, infiltration with 30 ml is sufficient, though anything up to 100 ml can be injected before the incision is made or as a supplementary treatment in the event of bleeding during or after conisation. Judging from our experience, re-infiltration will effectively control postoperative bleeding Rarely it is necessary to supplement such re-infiltration by Sorbacel tamponade in the cavity or by suturing The technique gives excellent results with good anatomic and functional recovery of the cervix (Kullander and Wehlin, 1965)

The cervix is preferably infiltrated from a few points in the circumference of the portio outside visible changes round the external os. Two deep sutures of the cervical branches of the uterine arteries in the lateral fornices promote hæmostasis and together with a claw forceps in the anterior and posterior part of the portio they provide excellent handles for infiltration and

conisation. Staining of the portio with Lugol's solution is best done after infiltration. Before the cone is removed it is also advisable to place a small indicator suture at the 12 o'clock position in the portio so that any pathological changes can be correlated with the gross findings before the operation. After routine curettage of the corpus microcrystalline sulpha powder is applied in the cavity after conisation. The cavity is not packed or electrocoagulated (Kullander 1965)

Since this investigation we have always used Octapressin prior to conisation (more than 200 cases) and, like Burghardt (1963) we have found it efficacious and without side effects.

Octapressin has also proved useful for reducing the blood loss in association with other gynaecological and obstetric operations. It can be used instead of epinephrine as a supplement to all types of local anaesthetic agents. Like Hochuli (1962) and Hochuli and Käser (1962) we have found the Octapressin infiltration to be particularly useful in conservative myomectomy and in episiotomy as an additive in local anesthetics.

SUMMARY

After infiltration of the portio with Octapressin (phenylalanine lysine vasopressin) or epinephrine cold-knife conisation can be performed in a bloodless field, suturing is unnecessary and the cosmetic results are excellent.

Comparison between epinephrine and Octapressin regarding their value in association with cold-knife conisation of the portio showed no difference in hæmostatic effect. Octapressin was however less often followed by untoward cardiovascular reactions

Infiltration with Octapressin also was used with advantage to reduce the blood loss in certain other gynæcological and obstetric operations.

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THE RISK OF DEVELOPMENT OF UTERINE CANCER IN PATIENTS WITH BREAST CANCER AFTER LONG TERM TREATMENT WITH ŒSTROGEN IN MASSIVE DOSES

BY

ODDMUND KOLLER

The question of the possible carcinogenic effect of œstrogen has recently been the subject of extensive reviews by Larson (1954) and Bishop (1960). Both of them list a report from the A.M.A. Committee on Therapeutic Trials stating that no cases of genital cancer occurred in over 1 000 women with inoperable breast cancer treated for 3 months to 2 years with massive doses of œstrogen. Larson (1954) adds that the patients have not been followed long enough to provide proof against the carcinogenic action of œstrogen.

Material

At the Norwegian Radium Hospital in the period 1949–1961 a total of 55 patients with inoperable breast cancer have been treated with massive doses of œstrogen for 1–9 years and observed for 3–13 years, representing 172 years of treatment and 347 years of observation.

Table I shows the distribution of the patients by years of treatment and observation.

The ages of the patients ranged between 44 and 87 years, with an average of 68 years.

Usually diethylstilboestrol was administered continuously in doses of 5 mg three times daily, but in 5 cases ethinyloestradiol was given in doses of 0.5 mg three times daily. In half of the patients, because of side-effects, the doses had to be reduced to 2

Table I *Years of Massive Œstrogen Treatment*

Years of observation									
13	2								
12	2								
11									
10	1							1	
9				2	2	1			1
8		1	1				1		
7	1	3			1		1		
6	2	1	2	1	2	1			
5	3	3	1	1	2				
4	1	2	2	3					
3	1	3	3						
	1	2	3	4	5	6	7	8	9

or 1 tablet a day and sometimes short drugfree intervals were necessary Uterine bleeding was reported in 19 patients between 54 and 79 years of age with an average of 66 years In 5 of these cases, where the bleeding was not of the ordinary œstrogendependant type, diagnostic curettage was performed.

In one of the cases the curettage revealed atypical hyperplasia of the endometrium with suspicion of infiltration.

2430/56 A. E. J 61 years of age. Nulligravida. Menopause at the age of 49. She was referred to the Norwegian Radium Hospital for inoperable breast cancer on the left side and treated with X-ray and tele-radium followed by hormone therapy She received diaethylstilbestrol 5 mg three times daily with good response and was operated on 6 months afterwards. Histological examination of the specimen showed residual carcinoma and lymph node metastases. After additional X-ray therapy œstrogen treatment was continued with full doses for 5 years Because of vaginal bleeding she was referred to the gynaecological department of the local hospital where a curettage was performed. Histological examination at the pathological laboratory of the Norwegian Radium Hospital showed adenomatous hyperplasia of the endometrium and in addition epithelium with more irregular papillomatous growth and nuclear abnormalities suspicious of infiltrative growth. Hysterectomy with salpingo- oophorectomy was performed. Histological examination of the specimen showed mostly simple adenomatous hyperplasia without nuclear abnormalities. In one small area (1×1 cm) however the epithelium showed a more papillomatous growth pattern and some nuclear abnormalities but no signs of infiltration. The pathologist concluded that the suspicious findings most likely were from a solitary papilloma. The patient died postoperatively from coronary thrombosis.

Discussion

The type of woman treated with oestrogen in this series is highly selected and cannot be claimed to be representative of a normal population. There is however no evidence to indicate that the endometrium of patients with breast cancer reacts in a way different from that of normal women.

Most of the women had passed the menopause several years before oestrogen treatment was started and the fact that only 19 of 55 patients developed uterine bleeding may indicate reduced sensitivity of the endometrium at this age.

There was, however no significant difference between the age of those patients who developed bleeding and those who did not.

Even with the reservations mentioned the result of the examination seems to indicate that the risk of development of endometrial cancer after long term oestrogen treatment, if present, is low

SUMMARY

A series is presented of 55 patients with inoperable breast cancer treated with massive doses of oestrogen for 1 to 9 years and observed for 3 to 13 years, representing 172 years of treatment and 347 years of observation.

In only one of the patients there was any suspicion of uterine malignancy The patient had been treated for 5 years when a curettage showed atypical hyperplasia of the endometrium with suspicious infiltration. This finding, however could not be confirmed at the histological examination of the total uterus after hysterectomy

The study supports the contention that long term oestrogen treatment does not involve any appreciable risk of uterine cancer development.

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GRANULOSA AND THECA CELL TUMOURS AND GENITAL CANCER

BY

ODDMUND KOLLER

Recent evidence concerning the beneficial effect of long-term steroid substitution therapy in postmenopausal women has made the question of the possible carcinogenic effect of oestrogens a very crucial one. One of the most important arguments for such carcinogenic effect has been the claim that a disproportionately high number of oestrogen-producing ovarian tumours is associated with carcinoma of the uterine corpus This is interpreted as a result of prolonged and unopposed oestrogen stimulation of the endometrium

The reported incidence of endometrial carcinoma in patients with feminizing tumours shows wide variations Dockerty and Mussey (1951) found 16 in a series of 87 cases Kottmeier (1952) found 4 in 98 cases Sjostedt and Wahlén (1961) 4 in 157 cases The last authors report on granulosa-cell tumours only Larson (1954) in a compilation of 919 reported cases of feminizing tumours found 44 cases of endometrial cancer He stated that the occurrence of endometrial carcinoma in the premenopausal woman with an ovarian feminizing tumour is probably a chance association, but the association of these two conditions in the postmenopausal woman is too frequent to be due to chance. Emge (1953) collected information on a total of 753 feminizing tumours from 40 teaching centres in the U.S.A. and Canada. He noted that the frequency of simultaneous occurrence of tumours decreased progressively as the size of the sample grew Of the total series 25 (3 3 per cent) had endometrial carcinoma.

Emge stated, According to the Law of Large Numbers the probability is that with a continued increase in the size of the sample these figures would ultimately level off somewhere between 1.5 and 2.5 per cent, which is quite close to the predictable incidence for ovarian tumours and uterine cancer occurring si multaneously by chance

In this paper a series of granulosa- and theca-cell tumours derived from a population with known cancer incidence will be presented and previous reports discussed.

Material

The series comprises 52 cases of granulosa- and theca-cell ova rian tumours which includes all cases treated at the Norwegian Radium Hospital from its start in 1932 to 1963. Until 1947 only 4 cases had been treated, but from 1948 the annual number of new cases has been fairly constant, with an average of three. The incidence of granulosa- and theca-cell tumours in Norway is not known, because those considered not malignant may not have been reported to the Cancer Registry Based on the information from the Cancer Registry and from the pathological laboratory of the Norwegian Radium Hospital about 15 cases a year may be a resonable estimate. Consequently the series of cases ad mitted to the Norwegian Radium Hospital is a very selected one and the selection presumably must have been on the basis of ma lignancy of the ovarian tumour or because of association with other malignant tumours treated at the Norwegian Radium Hos pital. The largest groups of these are in decreasing size cervical cancer cancer of the breast and endometrial cancer A large proportion of the patients underwent surgical procedures which should ha e revealed ovarian tumours.

Fifteen of the 52 patients had never been pregnant.

Table I shows the type of tumour by age.

Irregular agmal bleeding was reported in the history of 33 patients and in 25 of these there were also histological or cytological findings suggestive of abnormal oestrogen stimulation.

In 10 of the patients from the early part of the series neither curettage nor hysterectomy had been performed. None of them

Table I. *Type of Tumour by Age*
(The number of these tumours which were malignant is given in parentheses)

	39	49	-59	-69	-79	Total Number
Granulosa-cell	2	5	11	23	0	41 (15)
ovarian tumours	(1)	(3)	(3)	(8)	0	
Theca-cell		2	6	2	1	11 (3)
ovarian tumours		(1)	(2)			
Total	2	7	17	25	1	52 (18)
	(1)	(4)	(5)	(8)		

developed later symptoms indicating involvement of the endometrium

In 3 of the patients the uterus had been removed prior to the diagnosis of the ovarian tumour In one of these cases the operation had been performed because of benign uterine polyps when the patient was aged 55 five years past menopause Her ovarian tumour was diagnosed when she was 66 years of age The indication for hysterectomy in the remaining 2 cases was endometrial carcinoma

492/47 M. K. K. After 5 normal pregnancies this patient had the menopause at the age of 46 13 years later she noticed vaginal spotting A curettage showed adenocarcinoma and a vaginal hysterectomy was performed, followed by intravaginal radium treatment. She had frequent follow-up examinations for 4 years and then returned 2 years later because she had noticed enlargement of the abdomen. Pelvic examination showed a cystic tumour Bilateral salpingo-oophorectomy was performed and the histological examination revealed a benign granulosa-cell tumour of the right ovary At the previous vaginal hysterectomy 6 years ago the ovary had been reported normal by inspection. Postoperatively X-ray treatment was given and the patient has been well until her last report in July 1964

907/49 M. A. C. After 2 pregnancies the patient had the menopause at the age of 40. Ten years later she developed vaginal bleeding. A curettage showed adenocarcinoma and the uterus was removed by a laparotomy No postoperative irradiation was given. She was then quite well until 15 years later when she experienced difficulty in mi turition. A pelvic tumour was diagnosed and a laparotomy revealed extensive tumour infiltration which could

not be removed completely. The pathologist considered the growth most likely to be granulosa-cell tumour. Postoperatively she had X-ray treatment and remained well for 6 years when she had a local recurrence. She died 2 years later and the autopsy confirmed the diagnosis of malignant granulosa-cell tumour.

A coexistent endometrial carcinoma was found in one of the cases.

4 7/46 A. L. A. Multigravida. At the age of 55 one year after the menopause she had been treated for carcinoma of the breast by radical surgery and post operative radiation. Eight months afterwards she noticed vaginal spotting and was referred to the Norwegian Radium Hospital where a curettage was performed. Histological examination showed cervical polyp and adenomatous hyperplasia of the endometrium. Because of continuing and increasing vaginal bleeding repeat curettage was done 15 months later and this time the histological examination was suggestive of malignancy. Total hysterectomy with salpingo-oophorectomy was performed. The histological examination revealed an adenocarcinoma of the endometrium and a granulosa-cell tumour of the left ovary. Postoperative X-ray treatment was given, and the patient has been well since then.

In 4 cases the histological examination of the endometrium showed adenomatous hyperplasi with nuclear abnormalities, compatible with a stage 0 lesion.

Discussion

Because of the large number of endometrial cancers treated at the Norwegian Radium Hospital one would expect coexisting endometrial carcinoma and feminizing ovarian tumours to be found with undue frequency in the series presented. Since only one case was found, this seems to indicate that the simultaneous occurrence of these tumours is rather rare. Perhaps more suggestive of a correlation is the finding of 4 cases of possible precancerous lesions. This fits with data reported by Kottmeier (1952) who in addition to the 4 cases of invasive cancer among 98 cases of granulosa- and theca-cell tumours found 9 cases with atypical hyperplasia of the endometrium. But because of the uncertain clinical significance of such histological findings, the 4 cases of

precancerous lesions in the present series, represent only circumstantial evidence for a correlation between endometrical cancer and feminizing ovarian tumours.

If the total life span of the 52 women is considered, rather than the time at the diagnosis of the ovarian tumours endometrial carcinoma occurred 3 times among the 52 patients one being in a patient who previously had had breast cancer No case of cancer or a precancerous lesion of the cervix was diagnosed. This is not the distribution of cancer types which is expected if the simultaneous occurrence of granulosa and theca-cell tumours and genital cancer is only a chance finding. In Norway breast cancer is nearly three times as frequent as cervical cancer which again is nearly twice as frequent as endometrial cancer (The Cancer Registry of Norway 1964) Sjöstedt and Wahlén (1961) among their 157 cases of granulosa-cell tumours occurring in a well-defined geographical area (south-west Sweden) found 4 cases of endometrial carcinoma, 2 cases of breast carcinoma and no cases of cervical cancer either at the time of operation or later These figures also show a disproportionately high number of endometrial carcinomas compared with cancer of the breast and the cervix.

Diddle (1952) collected from the literature 926 granulosa-cell tumours and 262 theca-cell tumours and found among them 72 cases of endometrial cancer 9 cases of breast cancer and 5 cases of cervical cancer one of the latter being in stage o. The cancer rates in the population from which these cases are derived, are not known but it is reasonable to assume that the ratio between the types of tumours mentioned do not differ very much from that in New York State, which again is very similar to the Norwegian figures. Based on this reasoning it may be safe to state that even if the tendency of granulosa and theca-cell tumours to be associated with endometrial carcinoma does not seem to be very strong, the relative correlation (compared with cancer of the breast and cervix) is quite significant.

The series presented from the Norwegian Radium Hospital showed a high ratio of nulligravidæ (30 per cent) In Diddle's compilation a third and in Sjöstedt and Wahlén's material 29 % of the patients were nulligravidæ. This may partly explain

the relatively few cases of cervical cancer but certainly not the low incidence of breast cancer which occurs relatively frequently among unmarried women.

If the correlation between granulosa- and theca-cell tumours and endometrial cancer was due to carcinogenic effect of oestrogen, we might have expected a carcinogenic effect on the breast as well. The mammary gland is also a target organ for oestrogen. It may perhaps be explained by postulating that granulosa- and theca-cell tumours produce oestrogens of sufficient amount and quality to induce endometrial, but not mammary cancer A more resonable explanation seems to me to be that a common constitutional factor predisposes both for ovarian and endometrial cancer cf in the series presented the 2 cases of endometrial cancer occurring long before any symptoms of a hormone-producing tumour

Larson (1954) suggested that the coexistence of feminizing ovarian tumours and endometrial cancer might be a manifestation of the high incidence of multiple malignancies seen in most cancer cases A special close correlation appears to exist between cancer of the ovary and cancer of the endometrium. In a series from the Norwegian Radium Hospital presented by Bergsjo (1962) 13 per cent had cancer in both organs. In the large majority of cases this must be interpreted as a spread from one area to another but there are also several cases with well established or probable simultaneous development of cancer in both areas.

Jackson and Dockerty (1957) reported a high incidence of endometrial cancer among patients with the Stein-Leventhal syndrome. The authors suggest that this correlation may be the result of a carcinogenic effect of oestrogen on the endometrium. According to Dorfman (1963) however the ovaries in patients with the Stein Leventhal syndrome seem to be deficient oestrogen formers as compared with normal ones The high incidence of endometrial carcinoma in these patients may more likely be interpreted as the effect of hormonal imbalance rather than of oestrogen stimulation. Hormonal imbalance is a common factor in a large variety of tumour inducing experimental procedures (Pincus, 1958)

SUMMARY

Based on a review of previous reports on the coexistence of granulosa and theca-cell tumours and genital cancer and on the findings in a series of 52 cases of granulosa and theca-cell tumours from the Norwegian Radium Hospital the following conclusions are drawn.

Granulosa- and theca-cell tumours show a significantly closer correlation with endometrial carcinoma than with carcinoma of the breast and carcinoma of the cervix.

This correlation may more likely be accounted for by a common constitutional factor predisposing to both types of growths than by a carcinogenic effect of oestrogen produced by the ovarian tumours.

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6 ALPHA METHYL 17 ALPHA HYDROXY PROGESTERONE ACETATE[1] AS A CHEMOTHERAPEUTIC AGENT IN ADENOCARCINOMA OF THE UTERUS

BY

J. B. BONTE, A. DROCHMANS, AND P. IDE

There is much scope for improvement in the treatment of endometrial carcinoma. Using conventional methods local recurrences and distant metastases are common. Primary treatment is often inadequate because of physical limitations. Thus the efficacy of radiation therapy may be impaired by obesity or surgical treatment may be contraindicated because of metabolic disorders. In all these circumstances effective chemotherapy would be a useful adjunct.

In particular hormonal therapy might be considered in this context. Pituitary-ovarian dysfunction accompanied by excessive oestrogen production seems to provoke endometrial carcinoma in predisposed patients. Many such patients have in addition some metabolic disorder.

Moreover it has been found that the endometrium, even in the case of postmenopausal carcinoma, retains a degree of hormone dependency. In these circumstances progesterone and progestogens offer the best chance of favourably influencing the growth of endometrial carcinoma, either directly in their administered form or as a break-down product, or indirectly through inhibition of the pituitary.

[1] PROVERA UPJOHN (Medroxyprogesterone)

SUMMARY

Based on a review of previous reports on the coexistence of granulosa and theca-cell tumours and genital cancer and on the findings in a series of 52 cases of granulosa and theca-cell tumours from the Norwegian Radium Hospital the following conclusions are drawn.

Granulosa and theca-cell tumours show a significantly closer correlation with endometrial carcinoma than with carcinoma of the breast and carcinoma of the cervix.

This correlation may more likely be accounted for by a common constitutional factor predisposing to both types of growths than by a carcinogenic effect of oestrogen produced by the ovarian tumours

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Material and Method

At present over 20 patients with some form of endometrial car cinoma are being treated by us with 6α-methyl 17α-hydroxy progesterone acetate. The patients are being closely followed up by regular gynaecological examinations supplemented by other methods of investigation, e.g. cystoscopy proctoscopy intravenous pyelography lymphography and other radiological tech niques. When the tumour is readily accessible, tissue specimens are obtained at regular intervals with th Novak curette or biopsy forceps for histochemical and histological examination. Exfolia tive vaginal cytology makes it possible to follow not only the course of the carcinomatous process but also the changes in the patient s hormonal balance.

The effect of treatment on the symptomatology (pain and blood loss) the patient s general condition and her body weight are also being followed. Careful observation is being kept for additional symptoms which may arise from the high dosage of hormones.

From this series we have selected three pairs of similar cases. The choice of cases and hormonal treatment used were such as to throw light on the mechanism of action of progestogens in endometrial carcinoma. The first group consisted of two cases of primary endometrial carcinoma of the corpus uteri, with advanced proliferative invasion of the true pelvis who had no previous treatment. The second group consisted of two patients who a few months after total radical hysterectomy for endometrial carcinoma, had developed a carcinomatous lesion in the vaginal vault.

The third group consisted of two cases of adenocarcinoma ori ginally localised to the cervix which, after adequate radiotherapy had developed local recurrence with extensive invasion of the paracervical tissues.

Results

Treatment with 6α-methyl 17 -hydroxyprogesterone was continuous. Some patients received oral medication (PROVERA – UPJOHN) at a daily dosage of 20–70 mg others received a total

Apart from pure progesterone it is chiefly progestogens of the 17 α-hydroxyprogesterone type that are administered in hormonal therapy of endometrial carcinoma. 17 α-hydroxyprogesterone caproate has a more protracted effect than pure progesterone. 17 α-hydroxyprogesterone acetate seems to have the same activity as the caproate but has the considerable additional advantage of retaining a high degree of efficacy when administered orally. Methylation of this progestogen, while leaving intact its qualitative biological action, quantitatively increases its effect without producing secondary endocrine effects. 6 α-methyl 17 α-hydroxyprogesterone acetate moreover has a protracted effect when administered parenterally and is highly effective orally.

In an extensive survey of the literature, we have been able to collect 61 cases all except one relating to postmenopausal patients with endometrial carcinoma originating in the uterine body.

In 26 patients progesterone therapy was the primary treatment of the endometrial carcinoma confined to the uterus. The remaining 35 patients had previously received surgical treatment or radiotherapy or a combination of both, and were not given hormonal therapy until subsequent dissemination of metastatic growth (usually in the lungs) had occurred.

Wide histological variations were found in this review. The results obtained by progesterone therapy were evaluated on different bases: the criteria were strictly clinical in 31 cases, strictly histological in 19 and anatomical-clinical in the remaining 11 cases.

Arrest of growth and even regression of the primary and secondary tumours and the accompanying metrorrhagia were observed in over 50 per cent of cases.

In half the cases the carcinomatous tissue showed histological changes chiefly characterized by the appearance or intensification of the features of maturation and metaplasia, and by oedema and decidual change in the stroma.

In the light of these observations it seems useful to trace the factors which determine the hormone dependency of endometrial carcinomata; at the same time a histological approach could be made to locate the point of action of progestogens on this carcinoma tissue.

COLOUR PLATES

of 500–1 000 mg weekly divided into several intramuscular injections (DEPO-PROVERA – UPJOHN) At the dosages indicated, neither mode of administration caused any side-effect even after protracted therapy

Group I

The first patient with extensive endometrial carcinoma showed mainly vaginal and vulvar invasion. Histological examination of tissue from the uterine cavity and from the vaginal nodules disclosed virtually identical features of anaplastic carcinoma. The cells were of exceedingly abnormal shape, with little cytoplasm and numerous mitoses they proliferated diffusely into the stroma, were seldom arranged in clusters and were nowhere arranged in a glandular structure. Cytochemical examination showed that the carcinoma tissue displayed no muco-polysaccharide localizations The patient was treated by twice weekly injections of 250 mg medroxyprogesterone intramusculary Despite therapy there was surprisingly rapid dissemination, both locally and at a distance the patient was very ill and confined to bed. The supra clavicular and inguinal glands showed pronounced adenopathy and osseous and pulmonary metastases developed. Hormonal therapy was continued until the patient s death.

Carcinoma tissue obtained by biopsy from both the vagina and the uterus showed radical histological and cytochemical changes even after 14 days of medroxyprogesterone therapy The cytoplasm of the endometrial cells unmistakably increased in volume, and the nuclei seemed pyknotic The young stroma showed distinct decidual appearances. The endometrial cells showed local arrangement in groups.

Some of these cell groups developed a lumen and even showed an unmistakable glandular structure. Some underwent metaplasia to acanthomatous structures Although the majority of cells were free of muco-polysaccharide, PAS staining disclosed intensive muco-polysaccharide accumulation in a few cells

The other patient in this pair had extensive primary endometrial carcinoma the corpus uteri was obviously enlarged and adherent to the two lateral pelvic walls. The carcinoma tissue showed a distinct glandular structure with closely packed cell groups

COLOUR PLATES

1



2



Fig 1 Prior to Medroxyprogesterone therapy ulcerated vaginal recurrence of endometrial adenocarcinoma. H.E.S staining. X210.

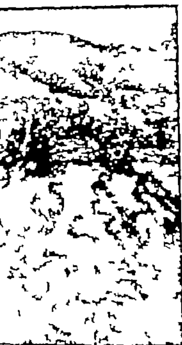
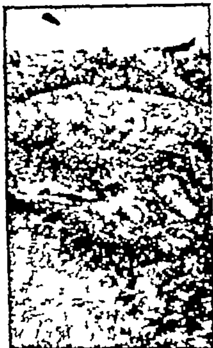
Fig Prior t Medroxyprogesterone therapy massive accumulation of muco-polysaccharide in adenocarcinoma cells. P.A.S. staining ×420

Fig. 3. After 2 weeks of Medroxyprogesterone therapy repressed glandular structures covered by developing stroma and normal vaginal epithelium H E S staining ×2 0

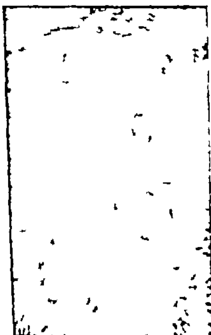
Fig. 4. After 2 weeks of Medroxyprogesterone therapy regression of muco-polysaccharide in adenocarcinoma cells P.A S staining ×420

Fig 5. After 4 weeks of Medroxyprogesterone therapy scanty glandular structures packed in fibrous stroma and covered by normal vaginal epithelium H E.S. staining × 0.

Fig 6 After 4 weeks of Medroxyprogesterone therapy atrophic glandular structures with marked regression of muco-polysaccharide P.A S staining ×420

5

6

1



2



Fig. 1 Prior to Medroxyprogesterone therapy ulcerated vaginal recurrence of endometrial adenocarcinoma. H.E.S staining ×210

Fig. 2 Prior to Medroxyprogesterone therapy massive accumulation of muco-polysaccharide in adenocarcinoma cells. P.A.S. staining ×420

Fig. 3. After 2 weeks of Medroxyprogesterone therapy regressed glandular structures covered by developing stroma and normal vaginal epithelium H.E.S staining × [illegible]

Fig. 4. After 2 weeks of Medroxyprogesterone therapy regression of muco-polysaccharide in adenocarcinoma cells P.A.S staining. ×4[illegible]0

Fig. 5 After 4 weeks of Medroxyprogesterone therapy scanty glandular structures packed in fibrous stroma and covered by normal vaginal epithelium H.E.S staining ×[illegible]

Fig. 6 After 4 weeks of Medroxyprogesterone therapy atrophic glandular structures with marked regression of muco-polysaccharide P.A.S staining [illegible]4[illegible]0

without discernible intermediate stroma the endometrial cells were typically cylindrical, very often with stratification so that the glandular lumen seemed very narrow In a few cells, microgranular muco-polysaccharide accumulation was found. The patient received 20 mg medroxyprogesterone orally per day

Although the carcinomatous invasion in the true pelvis seemed arrested, progressive nodular and probably metastatic hepatomegaly soon occurred. The patient died a month later Histological and cytochemical examinations disclosed progressive changes in the carcinoma tissue. The stroma developed quickly became highly cellular and sometimes even fibrous. The endometrial cells and their nuclei assumed irregular shapes. The cells formed detached groups retaining only a vague glandular structure and were found scattered in the stroma. The muco-polysaccharide localization did not seem to be influenced by the hormonal therapy

Group II

In the second experimental group, two patients were treated with medroxyprogesterone (750–1.000 mg weekly) for recurrence in the vaginal vault of an endometrial carcinoma treated by surgery

The first patient had undergone total radical hysterectomy with additional lymphadenectomy which had disclosed no metastatic adenopathy The tumour at the time seemed moderately differentiated. Three months later the woman developed an infiltration of the right vaginal angle with spread into the vesicovaginal septum, taking the form of a soft ulcerative tumour

Cytoscopy showed elevation of the bladder floor The carcinoma tissue had a glandulopapillary appearance produced by cylindrical cells with a tendency to stratification and abundant mitoses, surrounded by scanty stroma (Fig. 1) Cytochemical examination disclosed that all glandular cells even those in mitosis possessed an unmistakable accumulation of muco-polysaccharide at the base (Fig. 2) After 2 weeks hormonal therapy the vaginal tumour appeared to be appreciably less active. Beneath a normal vaginal epithelium a highly cellular fibrous stroma was developing, which embraced normal glandular structures

rounding the degenerative foci. Muco-polysaccharide occurred in all cells but especially the large round cells and the acanthomatous foci.

Group III

The third group consisted of two patients with a recurrence following radio-therapy of an endocervical glandular epithelioma. Both patients were treated for several months with oral medroxyprogesterone (500 mg evenly distributed over a week)

The first patient had received radio-therapy two years previously (pelvic irradiation with 4.000 r Telecobalt supplemented by intracavitary radium therapy) for an adenocarcinoma of the cervix uteri.

The recurrence occurred in the form of a plum-sized tumour in and above the vaginal vault, infiltrating the cervix and adherent to both lateral pelvic walls. While the primary tumour had been described as a glandular epithelioma, the recurrence showed diffuse infiltration of the stroma by malignant cells. After 3–5 weeks hormonal therapy the tumour disappeared and the cervix uteri could be reidentified as a fibrous stump enclosed in vaginal adhesions.

Tissue obtained by biopsy was submitted to histological examination and was found to consist entirely of connective tissue.

The second patient had two courses of radio-therapy within a 3 year period. The original tumour—an intracervical glandular epithelioma—was exclusively treated by radio-therapy (pelvic Telecobalt irradiation up to 4.000 r tumour dose supplemented by intracavitary application of radioactive Cobalt) A local relapse was observed two years later with the same histological features It was again treated by complete Telecobalt therapy

After a remission of 6 months, suspect tissue was obtained when the vaginal adhesions were broken down. Histological examination revealed florid carcinomatous tissue of the glandulo-papillary type. Cylindrical cells closely packed and with a minimal stroma reaction lined glandular ducts Considerable muco-polysaccharide accumulations existed, both intracellularly (vacuoles) and extracellularly (glandular lumina) Oral medroxyprogesterone therapy (500 mg per week) was instituted and has

lined with a single layer of small cells without mitotic pattern (Fig 3) While the cylindrical cells showed the same or even a larger amount of muco-polysaccharide (Fig 4) Two weeks later only a small flat area of vaginal induration persisted. A biopsy was taken from this induration through intact vaginal epithelium, atrophic glandular structures were found amidst a fibrous stroma (Fig 5)

Cytochemical examination no longer showed any muco-poly saccharide localization (Fig. 6) After 6 weeks medroxyprogesterone therapy a well-placed transmucosal biopsy disclosed no glandular tissue. After 10 weeks treatment gynæcological examination showed no abnormality The vaginal vault and vesico-vaginal septum were no longer infiltrated it was no longer either possible or necessary to obtain further tissue specimens for histological examination.

In the second patient an orange-sized soft tumour was found high in the vaginal vault 18 months after a simple total radical hysterectomy The tumour was partly ulcerated and adherent to the two lateral pelvic walls particularly on the right side. Moderate vaginal blood loss was the only recognizable symptom. The carcinoma tissue consisted of massive cell groups without any lumen but sometimes of glandular appearance embedded in a well-developed not very cellular stroma. The majority of cells contained very little muco-polysaccharide if any although there were moderate local muco-polysaccharide accumulations In the course of 5 months medroxyprogesterone treatment the patient was examined, initially twice weekly and later monthly and serial biopsies were performed.

Her general condition was excellent a slight weight gain was recorded. Vaginal blood loss was considerably reduced but did not stop completely Repeated gynæcological examinations showed arrest of tumour growth and spread.

Histological examination showed that the cells had become rounder and clearer but were still arranged in compact cell masses without lumen. Some cell masses had been metaplastically transformed into acanthomatous foci. Other cell masses showed central or total degeneration. The stroma initially showed regression but then increased and formed fibrous envelopes sur

4. The histological features of the carcinomatous tissue have no apparent effect on the clinical response to hormonal therapy but do influence the course of the tissue reaction. Poorly differentiated forms respond to progesterone by developing pronounced glandular structures by metaplasia to acanthomatous foci and sometimes by degeneration. the stroma undergoes decidualization and then sometimes fibrosis.

   Progesterone sometimes causes a differentiated endometrial carcinoma to be transformed by metaplasia into the acanthomatous type (usually degenerative changes are associated with development and fibrosis of the stroma)

5. (6 α-methyl- 17 α-hydroxyprogesterone acetate) PROVERA – UPJOHN has an effect on endometrial carcinoma both after parenteral and after oral administration. The histological changes occur early

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been continued for 9 months The globular corpus uteri, previously obscured by a pronounced parametrial and high vaginal infiltration, is now well-defined and mobilized.

The patient s general condition is very good the weight, which was falling became stable and is now showing a gradual rise. Vaginal blood loss has become sporadic. Repeated histological examinations during the course of medroxyprogesterone therapy have indicated pronounced stratification of the glandular structures with progressive narrowing of the lumen and epithelial metaplasia to extensive acanthomatous foci As in the original specimens the stromal reaction has remained minimal.

PAS staining has shown diminution of the muco-polysaccharide accumulation with the advance of metaplasia.

SUMMARY

To illustrate a larger experimental series to be described in detail later we have carried out a careful clinical study of medroxyprogesterone therapy in six cases of endometrial carcinoma. From our findings we can formulate hypotheses which may be useful as a guide to further investigations into hormonal therapy of endometrial carcinoma.

1 The extent of local spread of the carcinoma, as well as its extrapelvic spread seem to determine the ultimate course of progesterone therapy
Despite hormonal treatment, a very large and widespread endometrial carcinoma leads to a fatal issue before the (histologically demonstrable) regression can become clinically effective.

2 Intracervical forms of glandular epithelioma respond to progesterone therapy to the same extent as do endometrial tumours.

3 Preceding surgical treatment or radiotherapy does not seem to interfere with the hormone dependency of endometrial carcinoma.

Lindberg, S. and Törnqvist, A. Acta obst. et gynec. Scandinav. 43 131 1964

From the Department of Obstetrics and Gynecology (Professor Sam Brody)
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Sahlgren's Hospital University of Göteborg, Sweden

THE INHIBITORY EFFECT OF AMINOGUANIDINE ON HISTAMINE CATABOLISM IN HUMAN PREGNANCY

BY

S. LINDBERG AND A. TÖRNQVIST

In a previous study on the elimination of ^{14}C labelled histamine from the blood (Lindberg, 1963 a) it was found that the concentration of the histamine in arterial blood of pregnant women was approximately 50 per cent lower than that of non-pregnant women. As factors such as alterations in the blood volume and cardiac output during pregnancy were considered to be of minor importance in these investigations, it was suggested that the difference in arterial blood concentrations was due to more efficient histamine inactivation in pregnant subjects. In addition, metabolic studies carried out in the same cases provided conclusive evidence for the importance of diamine oxidase (histaminase) in the inactivation of histamine in human pregnancy. This phenomenon is well known from various studies *in vitro* but has hitherto not been demonstrated *in vivo*.

However, it was felt that further knowledge about the histaminolytic activity *in vivo* during human pregnancy could be gained from studies of the effects of aminoguanidine. Administration of aminoguanidine sulphate in proper dosage to non-pregnant humans has been shown to completely inhibit diamine oxidase (Lindell, Nilsson, Roos and Westling, 1960). It has also been given to pregnant women without any obvious untoward effects on either the mothers or the foetuses (Bjurö, Lindberg and Westling, 1964). However, in this study only the urinary excretion of non-labelled histamine was followed.

Lindberg, S. and Törnqvist, A. Acta obst. et gynec. Scandinav. 45: 131, 1966

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BY

S. LINDBERG AND Å. TÖRNQVIST

In a previous study on the elimination of ^{14}C-labelled histamine from the blood (Lindberg, 1963 a) it was found that the concentration of the histamine in arterial blood of pregnant women was approximately 50 per cent lower than that of non-pregnant women. As factors such as alterations in the blood volume and cardiac output during pregnancy were considered to be of minor importance in these investigations, it was suggested that the difference in arterial blood concentrations was due to more efficient histamine inactivation in pregnant subjects. In addition metabolic studies carried out in the same cases provided conclusive evidence for the importance of diamine oxidase (histaminase) in the inactivation of histamine in human pregnancy. This phenomenon is well known from various studies *in vitro* but has hitherto not been demonstrated *in vivo*.

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Table II. *Blood Concentration of ^{14}C-Histamine at Various Times after Start of Infusion. Values Refer to Counts per Minute of Pipsylhistamine per ml Blood*

(A=Brachial Artery B=Cubital Vein, C=Uterine Vein)

Time axis: 10, 20, 30, 40 min

Case		Values (in order along time axis)
1.	A	49, 49
	B	40, 43
	C	8
	A	6.0, 65
	B	6.1, 58
	C	44, 53
3	A	97, 87
	B	77, 67
	C	78, 8
4	A	79, 80
	B	66
	C	45, 6, 77
5.	A	71, 75
	B	59, 59
	C	53, 4
6.	A	7
	B	10, .0
	C	69, 74

been devised by Schayer and co-workers and carried out in the present study as described earlier (Lindberg, 1963 a). The following substances were assayed histamine 1-methyl-4 (β-aminoethyl)-imidazole (methylhistamine) 1-methylimidazole-4 acetic acid (methylimidazoleacetic acid) and imidazole-4(5) acetic acid (imidazoleacetic acid). Only a brief summary of the procedures will be given here. Each substance to be assayed is allowed to mix with the corresponding non-radioactive compound (carrier) in amounts equivalent to 206 mg of its picrate. The presence of carrier substance makes possible an extraction procedure by ordinary chemical methods as the ratio between the radioactive compound and its carrier remains unchanged. A preliminary extraction is then performed using butanol as solvent

Table I *Description of Cases and Infusion Rates*

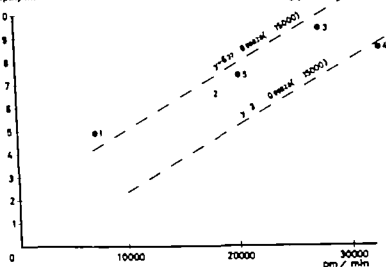
Case	Age Years	Duration of Pregnancy	Body Weight kg	Dose of ^{14}C-Histamine cpm/min	Dose per kg Body Weight cpm/min/kg
1	27	18 weeks	74	7700	105
2	39	17	65	17700	270
3	38	16	89	[illegible]	305
4	40	15	71	32500	460
5	43	15	75	[illegible]	[illegible]
6	43	16	53	25000	470

Material and methods

Six healthy pregnant women were studied. The patients had been admitted to the obstetrical department for legal abortion and sterilization. Information regarding the patients is given in Table I

Blood samples were obtained during laparotomy as described in detail previously (Lindberg, 1963 a) Each patient received aminoguanidine sulphate (Eastman Organic Chemicals) subcutaneously in 2 doses of 0.1 mg/kg body weight, given 2 hrs and ½ hr respectively before the operation. The labelled ^{14}C histamine (The Radiochemical Centre Amersham, England) was infused intravenously during general anaesthesia. The infusion rate was maintained at 0.1–0.3 μg histamine base per kg body weight per minute by means of a motor-driven syringe No unexpected changes in pulse rate or blood pressure were observed during the infusion Blood samples were withdrawn at steady state by direct puncture of a superficial cubital vein and a uterine vein. Arterial blood was obtained from the brachial artery through a previously inserted polythene catheter As far as possible blood was collected simultaneously from each vessel The blood sample to be assayed for ^{14}C histamine was immediately mixed with a known volume of non isotopic carrier substance The remaining samples were stored with HCl ready for subsequent carrier addition and extraction for the determination of metabolites.

The analysis of the ^{14}C-containing compounds in blood has



ARTERIAL BLOOD CONCENTRATION OF ^{14}C-HISTAMINE RELATED TO THE RATE OF ^{14}C HISTAMINE INFUSION

alues are given in cpm per ml blood and cpm per minute

Fig 1.

Table III Blood Concentration of ^{14}C-Histamine in Various Vessels Values Expressed as per Cent of Arterial Concentration

Case	Brachial Art	Colonic Vein	Uterine Vein
	00	86	57
	00	96	78
3	00	78	86
4	00	00	63
5	00	8	65
6	00	94	67
Mean	00	89	69

compared with the corresponding levels in the venous samples collected. Table III is based on the experimental data of Table II For comparati e purposes the arterial values are taken as 100 and the venous values are expressed as a percentage of the arterial concentration. In all cases examined the concentration of

for histamine and chloroform as solvent for methylhistamine. The acids are adsorbed on an anion-exchange resin. All the compounds are crystallized as picrates. For the final determination of histamine and imidazoleacetic acid the picrates are converted to derivatives of p-iodophenylsulphonylchloride (pipsyl). All assays for imidazoleacetic acid are preceded by acid hydrolysis in order to liberate the ribose-bound fraction. As a rule all the samples investigated required at least three recrystallizations to ensure constant radioactivity. All samples were counted at infinite thickness in a flow counter (Nukab) with a background of 20–23 counts per minute. At least 2000 counts were recorded between recrystallizations. One microgram of the ^{14}C-histamine used in the investigation gave about 3000 cpm above background count when assayed in the form of the picrate.

Results

The concentrations of ^{14}C-histamine in cpm per ml of blood in all samples obtained at the indicated intervals after the start of infusion are shown in Table II. The values refer to pipsylhistamine. On no occasion was the infusion and sampling period longer than 40 minutes, as can be seen in the table.

The concentration of ^{14}C histamine in arterial blood is of primary interest. Its dependence upon infusion rate has been shown in a previous investigation (Lindberg, 1963 a) and is confirmed in the present series of cases. The relationship between arterial concentration and infusion rate of ^{14}C-histamine per minute is shown in Figure 1. The regression lines for the pregnant and non-pregnant series of the previous investigation are also given to allow a comparison with the present cases. As seen from Table II in five out of six cases the values obtained for the arterial concentration of ^{14}C-histamine seem to fall close to those of the non-pregnant series of the previous investigation whereas the sixth falls near the regression line of the pregnant series. There are insufficient cases in the present study to justify fitting a regression line for these observations.

In Table III the arterial concentration of ^{14}C-histamine is

Discussion

The arterial blood concentration of ^{14}C-histamine in the present series of pregnant women is definitely higher than in the previous study (Lindberg, 1963 a) in which the patients did not receive aminoguanidine. This provides additional evidence for the action of diamine oxidase during pregnancy aminoguanidine being a strong inhibitor of this enzyme (Lindell *et al* 1960) In one case (Case 4) however the arterial blood concentration was considerably lower than in the others. But due to the lack of imidazoleacetic acid in this case it may also be concluded that the aminoguanidine was not ineffective even in this case. Thus technical errors or strong methylation must not be excluded as conceivable causes of the low histamine concentration. The wide occurrence of imidazole N-methyl transferase in human tissues and the specific localization of diamine oxidase for instance to the placenta and myometrium during pregnancy has been pointed out in a recent paper (Lindberg, 1963 b) In an *in vitro* study on placental tissue at term (Lindberg, 1963 c) it was shown that the inhibition of diamine oxidase does not affect the total histaminolytic activity of the tissue owing to the methylation

A striking feature of the metabolites of ^{14}C histamine in the blood is the complete lack of imidazoleacetic acid, the predominant metabolite being methylhistamine This would appear to indicate complete inhibition by aminoguanidine in all four cases in which the metabolites were investigated. Thus the results also provide evidence for the presence of a potent active imidazole N-methyl transferase in pregnant subjects *in vivo* The methylhistamine has also been further oxidized to a considerable extent to MeImAA. That the ratio MeHi to MeImAA in the present study is the same as in the aforementioned study without aminoguanidine (Lindberg, 1963 a) can hardly be judged from the limited material It seems obvious, however that aminoguanidine had no effect on this oxidative process, which is in accordance with a previous report (Rothschild and Schaye 1958) A similar distribution of the metabolic products in the urine has been reported by Lindell *et al.* (1960)

Table IV *Blood Concentration of ¹⁴C Histamine and Its Metabolites. Values Are Given in cpm per ml Blood*

Case	Brach. Art.	Cub. Vein	Ut. Vein
HI	5.4	4.6	3.1
MeHI	3.5	3.9	3.7
MeImAA	1.6	–	2.0
ImAA	0	0	0
Sum	10.5	–	8.8

Case	Brach. Art.	Cub. Vein	Ut. Vein
HI	6.9	6.6	5.4
MeHI	3.4	3.7	3.7
MeImAA	2.4	3.0	2.0
ImAA	0	0	0
Sum	12.7	13.3	11.1

Case 3			
HI	10.1	–	8.7
MeHI	6.7	–	6.6
MeImAA	3.6	–	3.4
ImAA	0	–	0
Sum	20.4	–	18.7

Case 4			
HI	9.2	–	5.8
MeHI	6.3	–	7.6
MeImAA	4.6	–	3.4
ImAA	0	–	0
Sum	20.1	–	16.8

¹⁴C histamine was lower in the uterine vein than in the brachial artery, the values ranging from 57 to 86 per cent with a mean of 69 per cent. For cubital venous blood the corresponding mean value was 89 per cent, which is in good agreement with the results of previous studies (Lindberg, 1963 a).

In four cases the three principal metabolites were measured in arterial and venous blood. The results are presented in Table IV. All the values obtained for pipsyl compounds of histamine have been multiplied by a factor of 1.1 to make them comparable with the other compounds, which were measured as picrates. In each case the sum of the concentrations of histamine and its metabolites is lower in the uterine vein than in the brachial artery. An increase in the total metabolites in the uterine vein would have been expected as a result of the uterine histaminolysis. As in the previous study (Lindberg, 1963 a) this was not the fact in the present investigation either. A complete lack of imidazoleacetic acid was observed in all cases examined. Methylhistamine was the predominant methylated end-product

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ImAA	0	–	0	ImAA	0	–	0
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of histamine was higher than in a previous study in which aminoguanidine was not administered. The elimination of histamine from the blood by the pregnant uterus is obviously lower when aminoguanidine is given. A complete lack of imidazoleacetic acid in the circulating blood was observed. Methylhistamine was the predominant methylated end-product.

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after single injections of ^{14}C histamine in non pregnant subjects during treatment with aminoguanidine

Of special interest are the findings regarding the difference in ^{14}C histamine concentration in samples from the brachia artery and the uterine vein as the maternal part of the placenta has been considered as a source of diamine oxidase (Swan berg, 1950) In the previous work (Lindberg, 1963a) a elimination of 49 per cent of the circulating ^{14}C histamine durin passage through the uterus was found in pregnant subjects while in the non pregnant series the corresponding value wa 36 per cent In the present study the corresponding value fo aminoguanidine-treated pregnant subjects was 31 per cent, i.e of the same magnitude as in the non-pregnant group of th previous study This indicates that the imidazole N-methyl tran ferase has not succeeded, as a rule in replacing completely th inhibited diamine oxidase with respect to the total uterin histaminolytic activity

The present investigation has revealed that during huma pregnancy the two major enzyme systems diamine oxidase an imidazole N-methyl transferase are active in maintaining th (arterial) blood concentration of histamine at a lower level tha in non-pregnant subjects It seems likely that this phenomeno is also of importance for the histamine sensitivity in pregnan women since it has been shown that aminoguanidine will poten tiate the effect of histamine (Tornqvist, 1965) Thus the in creased diamine oxidase activity during pregnancy could be con sidered as a means of keeping the histamine response at a sui able level

SUMMARY

After administration of aminoguanidine the metabolism of ^{14}C labelled histamine was studied *in vivo* in 6 pregnant wome Blood samples were taken simultaneously from the brachi artery the cubital vein and the uterine vein during a continuou infusion of ^{14}C-histamine Judging by metabolic studies carrie out in 4 of the cases, the inhibitory effect of aminoguanidine wa complete In the present series the arterial blood concentratio



Fig. General view of the abdomen. Twin A is in the second cephalic presentation with head high. Twin B is in the second breech presentation. The head of twin B may be seen lying outside the contours of the uterus (upper left-hand corner of the film).

prematurely in 948 and 95 weighing 300 and 2,400 g respectively. Both are alive and well. During her present pregnancy in her second marriage there had been no pelvic pain or vaginal bleeding, but she had been more tired than in her previous pregnancies. Nevertheless, she had carried on in her job as ironer up to 6 weeks before term. Immediately before her admission her blood pressure had risen to 170/ 0, and albuminuria had appeared. The weight gain during the pregnancy now amounted to 6 kg. The

Felbo M and Fenger H J Acta obst et gynec Scandinav [illegible]

From the Royal Maternity Department B (Professor E Brandstrup) Rigshospitalet Copenhagen Denmark

COMBINED EXTRA AND INTRAUTERINE PREGNANCY CARRIED TO TERM

BY

M. FELBO AND H J FENGER

Combined extra and intrauterine pregnancy (syn. heterotopic pregnancy) is an obstetrical rarity which was reported for the first time by Duverney who observed it at autopsy in 1708. In a review of the world's literature up to 1926 however Novak collected 276 cases. According to Ludwig's analysis by 1935 the total had increased to 353 and in 1952 it was 415 (Zaran and Sy) In Vasicka and Grable's description of the condition in 1956 435 cases were listed Up to December 1963 another 87 cases have been added, bringing the total to 522. Interstitial pregnancies are not included. Out of this number of combined pregnancies there are only 11 cases in which the mother and both infants have survived the first months after delivery The first report of such a case was published in 1908 by Miller The others were published by Dubose (1915) Fejer and Henry (1949) Gilliland (1949) Nandi (1953) Loxton (1953) Parker (1955) Vasicka and Grable (1955) Chapman (1957) Hathaway and Vasquez (1961) and by Burkhart, Mule Begneaud and Kohen (1963) To these may be added a case from the Royal Maternity Department B Rigshospitalet, Copenhagen

Case Report (Rec No B 245/64)

The patient was a 42-year-old gravida III who was admitted 3 weeks before term because of oblique presentation, pre-eclampsia and anemia. She had never had pelvic infection. In her first marriage she had two children, born



Fig. 1. General view of the abdomen. Twin A is in the second cephalic presentation with head high. Twin B is in the second breech presentation. The head of twin B may be seen lying outside the contours of the uterus (upper left-hand corner of the film)

prematurely in 948 and 95 weighing 500 and 2400 g respectively. Both ar alive and well. During her present pregnancy in her second marriage, there had been no pelvic pain or vaginal bleeding, but she had been more tired than in her previous pregnancies. Nevertheless, she had carried on in her job as ironer up to 6 weeks before term. Immediately before her admission her blood pressure had risen to 170/ 0, and albuminuria had appeared. The weight gain during the pregnancy now amounted to 6 kg. The

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Fig. 3 a–b Photos of the twins immediately after birth and 7 months later

was performed, removing normal-looking placenta, weight 440 g, from small, intact cavity. During this procedure the patient grew ever more pale and her B.P. became unmeasurable. She reported only negligible pain without radiation. Therapy for shock was instituted immediately and the B.P. rose to mm Hg systolic, but blood had to be transfused rapidly to keep the B.P. normal. When digital examination under general anaesthesia was repeated, it was confirmed that the uterus had not ruptured, that it contained no septum, that there was only one cervix, and that the vagina was normal. The abdominal meteorism was increased, there was dullness laterally and the circumference of the abdomen had increased from 94 cm at delivery to 98 cm. After therapy for shock was instituted further X-ray



Fig. 2. Photo of the internal genitalia during the laparotomy. The well-contracted uterus is visible inferiorly on the right. In the centre there is the opened amniotic sac from the extrauterine pregnancy, the proximal part of the placenta anteriorly and the cord with the insertion into the membranes posteriorly. The distal part of the placenta is not visible on the photo. Superiorly in the membranes, and at the site of the left uterine cornu, there are bleeding vessels.

haemoglobin concentration was 7.6 g %. A suspicion of twins arose and this diagnosis was confirmed by X-rays (Fig. 1). During treatment with sedatives, diuretics, and 200 ml packed red cells her condition returned to normal, so there did not seem to be an indication for inducing labour before term.

Delivery. The membranes ruptured at term. As thi was not followed by labour pains, a Syntocinon-Efosin drip infusion was instituted. At the end of 18 hours, i.e. at 12.55 p.m. on Feb. 1964 she was delivered of a liveborn girl in the second regular occipital presentation (*vide infra*). Immediately after the delivery the well-contracted uterus could be palpated inferiorly on the right in the abdomen, which was distinctly distended. Heart sounds were audible in the epigastric region, but t was impossible to differentiate foetal parts. Under brief ether anaesthesia, intrauterine palpation

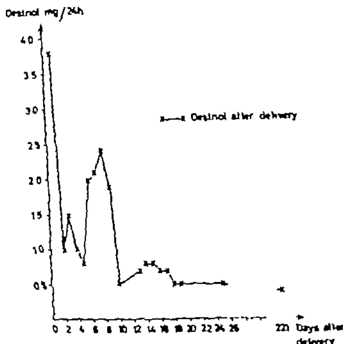
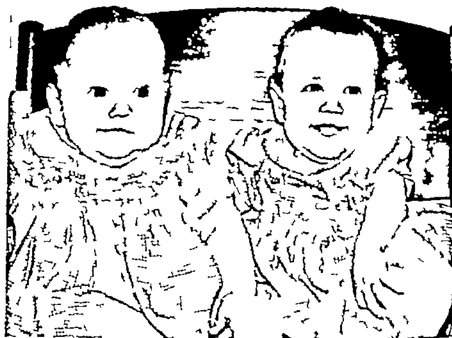


Fig. 4. Urinary excretion of oestriol postoperatively as function of time. An abrupt fall of the titr to normal values during the first 24 hours, followed by an increase until the 6th day. Thereafter again a decrease to 5 mg/24 h values on the oth day

part of the amniotic sac with the cord was removed without any technical difficulties. This part weighed 590 g. As far as the lower part of the placenta was concerned, the anatomical features were somewhat confused, and it was feared that its removal might give rise to uncontrollable bleeding. There were no signs of placental detachment, so it was decided to leave this part behind. At an estimate it weighed 500 g. After securing haemostasis, the uterus was fixed to the pelvic peritoneum, covering the placental remnant, and drain was established from the free peritoneal cavity. The total haemorrhage amounted to about 4 litres. In connection with the procedure 8 blood units of 500 ml each were transfused.

Postoperative course: At the end of the operation the systolic B.P. was 35



b

of the abdomen was obtained. This showed twin B in a very high transverse position.

As a ruptured uterus and a double uterus had been excluded, it was concluded that this must be an extrauterine pregnancy which had ruptured. Accordingly an inferior median laparotomy was performed.

Operative findings. The peritoneal ca ity was f lled with blood, estimated 3 litres. The f etus was lying free in the peritoneal cavity in the first transverse position. 100 minutes after the delivery of the first infant, another live girl was born. The widespread placenta was bipartite. Its upper part adhered to the left uterine cornu, where large vessels led to the lower part which spread out o er the left parametrium, lining the bottom of the cul-de-sac and extended to the lateral aspect of the true pelvis and the sigmoid colon. The membranes adhered, in the left side of the abdomen, to the omentum and to a single coil of the small intestine and the sigmoid, but was easy to mobilize. The cord was inserted 20 cm from the closest edge of the placenta. There was profuse bleeding from vessels in the left uterine cornu and at the marginal zone of the hole in the membranes (Fig. 2). There was no old bloodclots as a sign of bleeding during the pregnancy in the abdominal cavity. The upper part of the placenta as well as the greater

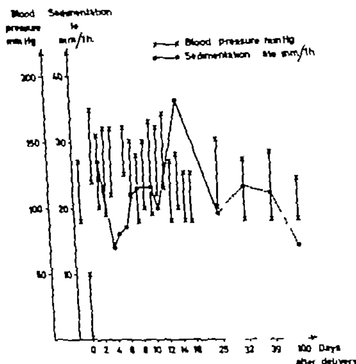


Fig. 6. Blood pressure and erythrocyte sedimentation rate in relation to time after the operation (on 7.2.64). Blood pressure on the day of the operation 35/90, after delivery of twin A 50/0. Postoperatively it was elevated until the 5th day. Erythrocyte sedimentation rate during the first 4 days after the operation between 5 and 36 mm/hour. Thereafter decreasing. 95 days after the operation 5 mm/hour.

occurred on the 3rd day. Daily determinations of oestriol by the method of Frandsen (Fig. 4) and of chorionic gonadotrophin by the method of Wide (Fig. 5) were carried out on 24-hour specimens of urine. Furthermore, to ascertain possible changes in the placenta, we determined the E.S.R., white cell counts, serum glutamic pyruvic acid (SGP) transaminases daily for days and thereafter at increasing intervals. Only the E.S.R. was elevated.

Gynecological examination on the 8th day revealed the uterus to be anteflexed, displaced to the left, well contracted, and partially fixed. The placental remnant was barely palpable inferiorly in the left side of the pouch of Douglas and above the left inguinal ligament. At the juncture the

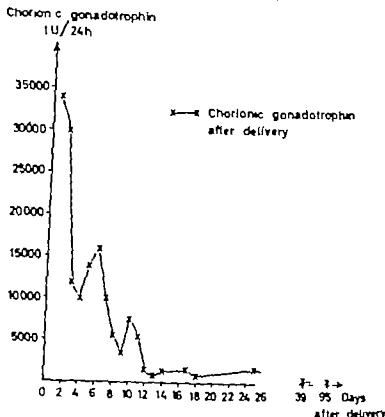


Fig 5. Urinary excretion of chorionic gonadotrophin postoperatively A transient fall in the titre during the first days was followed by an even decrease from 16 000 i u on the 6th day The titre on the 25th day was 1600 i u on the 39th day 270 i u and on the 95th day 5 i u.

mm Hg. An indwelling catheter was inserted. The urinary output was 450–500 ml/24 hours during the first 5 days and did not exceed 1000 ml until the 7th day The serum creatinine was maximally 1 4 mg/100 ml and returned to normal on the second day During the first 24 hours 800 ml of almost pure blood was discharged through the abdominal drain but after that there was only slight oozing. The drain was removed on the 6th day To prevent distension only parenteral nutrition was given for one week. By intravenous infusion she received 1000 ml human serum, 1000 ml blood 200 ml human albumin, electrolytes and glucose In addition broad-spectrum antibiotics were administered.

Investigations On the second day the B.P rose to 175/115 The pressure did not fall until the 5th day to 140/110 mm Hg (*Fig. 6*) There was no postoperative albuminuria. The maximum elevation of temperature to 38 C,

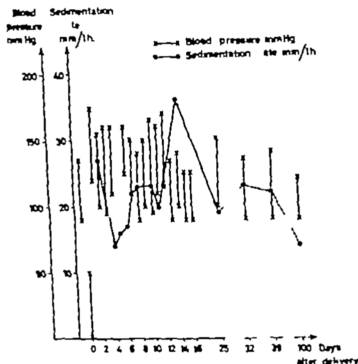


Fig 6 Blood pressure and erythrocyte sedimentation rate in relation to time after the operation (on 7 64) Blood pressure on the day of the operation 35/90, after delivery of twin A 50/0. Postoperatively it was elevated until the 13th day Erythrocyte sedimentation rate during the first 4 days after the operation between 5 and 36 mm/hour Thereafter decreasing 95 days after the operation 5 mm/hour

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oestriol values were 0.5 mg/24 h. The chorionic gonadotrophin values were still elevated.

At follow-up gynaecological examination 3 months after the operation, the placenta had not definitely decreased in size. The chorionic gonadotrophin values had returned to normal (below 5 I.u.) The patient weighed 3 kg more than at the time of discharge. Hb 14.0 g/100 ml, B.P. 120/90, ESR 14 mm/hr., W.B.C. 4,800/mm. Urine: No albumin.

Infants.

Twin A, a live girl weighing 2600 g and measuring 49 cm, was dysmature with a dry skin and reduced tone. She cried at once and was treated with glucose-saline subcutaneously. When discharged, on the 19th day, she was thriving and weighed 2900 g.

Twin B, a live girl of 3000 g and 50 cm in length, was delivered by laparotomy. She was very pale at birth, and there was no spontaneous respiration. The heart beat was slow. Not until 45 minutes post partum could the infant be transferred from the resuscitation table to an incubator. Apart from slight cerebral irritation during the first days, which was treated with phenobarbitone, the infant showed no abnormalities and gradually thrived on a 50 % formula nine times daily. Discharged on the 19th day weighing 2950 g.

Case Summary

A 42 year-old gravida III was admitted 3 weeks before term because of pre-eclampsia, oblique presentation, and anaemia. In 1948 and 1951 the patient had been delivered of premature infants weighing 1500 and 2400 g respectively. During her present pregnancy there had not been pain or bleeding at any time. The diagnosis of twins was confirmed by X-rays. Twin A, a live girl 2600 g/49 cm, was delivered without complications in the second occipital presentation. As the uterus thereafter was contracted and twin B difficult to palpate, intrauterine palpation was performed and the first placenta was removed manually. During this procedure the patient became shocked and this was immediately treated. After she had recovered from the shock, a repeated inspection and palpation were done, confirming that there was only one vagina, one cervix, and one empty, well contracted uterus. On the indication of a ruptured extrauterine pregnancy an inferior median laparotomy was carried out. From

the abdominal cavity twin B, an asphyxiated girl of 3000 g/50 cm, was delivered 100 min. after the birth of twin A.

This was apparently a case of an ovarian extrauterine pregnancy with a widespread, deep bipartite placenta situated on the left of the uterus and extending into the true pelvis. The amniotic sac and upper medial part of the placenta could be removed *in toto* (390 g) while the lower part of the placenta, estimated to weigh 300 g, was left behind after careful haemostasis. A drain was established from the free peritoneal cavity. In connection with the operation 4000 ml of blood was transfused. Postoperatively the case was complicated by paralytic ileus. On the 19th day the mother and infants were discharged to their home. The oestriol and chorionic gonadotrophin excretion from the placental remnant left behind were studied for 90 days after the operation.

Discussion

A *diagnosis* of combined pregnancy if made at an *early* stage, is based on the frequently recurring acute symptoms, due to the tubal localization of the extrauterine pregnancy.

The symptoms and signs of *late* combined pregnancies are of such a nature that the correct diagnosis is seldom made. As a rule, these patients have in the course of pregnancy had minor attacks of pain or other abdominal discomfort, sometimes accompanied by vaginal bleeding. The objective investigation as a rule reveals only twins. In our case the course was silent until the birth of twin A, and indeed the operation did not show any old blood clots in the peritoneal cavity. The left appendage was not observed, but according to the clinical features it must be presumed that the extrauterine pregnancy developed as an ovarian pregnancy with optimum nutritional conditions. In the 12 combined pregnancies which have so far resulted in live mothers and infants who have survived the first month after delivery the diagnosis has never been based on clinical examination alone, and only in 3 cases on X-ray examination prior to the birth of twin A (Vasicka and Grable, Burkhard *et al.* 1956, Hathaway *et al.* 1961). A foetal part outside the out

lines of the uterus confirmed the diagnosis of extrauterine pregnancy in the present case Had this caught our attention, the combined pregnancy could have been diagnosed on the basis of the general view (Fig 1) of the abdomen

Treatment

If combined pregnancy is diagnosed at an *early* stage of pregnancy the risk of intra-abdominal hæmorrhage indicates interruption of the extrauterine pregnancy In the case of *late* combined pregnancies where there is doubt regarding the viability of the fœtus because of its small size the patient should be admitted for observation Operative procedures are not indicated until major symptoms appear or labour is imminent. In order to avoid rupture of the extrauterine pregnancy leading to manifest shock or intra-abdominal fœtal death, the delivery of both infants should be by the abdominal route In 3 of the 12 cases the diagnosis was made before the delivery of twin A, and both the intra and extrauterine infant were delivered abdominally In the remaining 9 cases the diagnosis was not made until after the delivery of twin A. In Chapman s case Cæsarean section was carried out 2 weeks before term to deliver intrauterine twins. Bidigital vaginal examination and inspection had failed to disclose the cervix, so that a spontaneous vaginal delivery was considered out of the question. Classical Cæsarean section revealed only one intrauterine live infant. The incision was prolonged, giving access to the extrauterine pregnancy and another live infant was delivered. It was the extrauterine pregnancy which had displaced the cervix upward and forward so that it could not be palpated or seen. In the eight remaining cases the laparotomy was carried out as follows In three cases less than 24 hours after the birth of twin A in the others 2 24 35, 49 and 61 days after the delivery of twin A

In the present case no technical problems due to vascularity occurred during the removal of the upper part of the bipartite placenta The vascular supply of the distal part was not identified and as no signs of detachment of this part were observed in the course of the operation it was decided to leave it This is

in conformity with obstetrical practice in single abdominal pregnancies. Analysis of major series of this nature establishes the importance partly of *avoiding unnecessary manipulations and partly of not removing the placenta unless the afferent vessels easily lend themselves to ligation* It is stated, for instance by Charlewood and Culiner (1955) that out of 24 mothers with late extrauterine pregnancy in whom the placenta was removed 5 died, while no deaths occurred among 18 in whom the placenta was left behind. Hreshchyshyn, Bogen and Loughen (1961) analysing 101 cases from the period 1945–1957 recommend removal of the placenta in most cases but in 57 per cent this involved the necessity for hysterectomy A correspondingly high incidence of hysterectomy may be found in the series of Yahia and Montgomery (1956) and Dixon and Stewart (1960) comprising 10 and 8 patients respectively without any maternal mortality On the other hand, the morbidity and stay in hospital are considerably reduced if the placenta is removed. In late combined pregnancy Vasicka and Grable found fatal, postoperative haemorrhage in 2 out of 14 cases in which the placenta had been removed, while 2 out of 5 mothers died of haemorrhage, after the placenta had been left behind. However most of their patients are from the era prior to modern antibiotic and shock-combating therapy Drainage from the peritoneal cavity may presumably contribute to detecting late placental detachment as early as possible. In our case about 3/4 l of blood was discharged through the drain during the first 24 hours, and this probably contributed to shortening the duration of the intestinal paralysis.

Risk Involved in Combined Pregnancy

De oe and Pratt's analysis of the *maternal mortality* of combined pregnancies up to 1935 showed an incidence of 19 per cent. Since that time the risk has considerably decreased because of effective measures against shock and antibiotic therapy Vasicka and Grable report that from 1935 to 1947 it was 0.5 per cent and during the period 1936 to 1956 0.98 per cent. The highest risk for mothers with combined pregnancy is at an

lines of the uterus confirmed the diagnosis of extrauterine pregnancy in the present case. Had this caught our attention, the combined pregnancy could have been diagnosed on the basis of the general view (Fig. 1) of the abdomen.

Treatment

If combined pregnancy is diagnosed at an *early* stage of pregnancy, the risk of intra-abdominal hæmorrhage indicates interruption of the extrauterine pregnancy. In the case of *late* combined pregnancies where there is doubt regarding the viability of the fœtus because of its small size the patient should be admitted for observation. Operative procedures are not indicated until major symptoms appear or labour is imminent. In order to avoid rupture of the extrauterine pregnancy leading to manifest shock or intra-abdominal fœtal death, the delivery of both infants should be by the abdominal route. In 3 of the 12 cases the diagnosis was made before the delivery of twin A, and both the intra and extrauterine infant were delivered abdominally. In the remaining 9 cases the diagnosis was not made until after the delivery of twin A. In Chapman's case Cæsarean section was carried out 2 weeks before term to deliver intrauterine twins. Bidigital vaginal examination and inspection had failed to disclose the cervix so that a spontaneous vaginal delivery was considered out of the question. Classical Cæsarean section revealed only one intrauterine live infant. The incision was prolonged, giving access to the extrauterine pregnancy and another live infant was delivered. It was the extrauterine pregnancy which had displaced the cervix upward and forward, so that it could not be palpated or seen. In the eight remaining cases the laparotomy was carried out as follows. In three cases less than 24 hours after the birth of twin A, in the others 2, 24, 35, 49 and 61 days after the delivery of twin A.

In the present case no technical problems due to vascularity occurred during the removal of the upper part of the bipartite placenta. The vascular supply of the distal part was not identified, and as no signs of detachment of this part were observed in the course of the operation, it was decided to leave it. This is

Figs 4 and 5 show the alterations in the urinary excretion of oestriol and chorionic gonadotrophin during the first three months after the operation. Both curves show a parallel course with a transient fall during the first postoperative week, presumably reflecting the patient's poor general condition.

The oestriol values were 0.5 mg/24 h. on the 10th day. The chorionic gonadotrophin excretion was 1600 units on the 25th day and 260 units on the 39th day. Not until more than 3 months after the operation was the chorionic gonadotrophin titre below 25 I.U. Ware (1948) demonstrated a positive Friedman test 35 days after delivery in two cases where the entire placenta had been left behind in the abdominal cavity. In a similar case Weinberg (1958) found the Friedman test to become negative between the 26th and 54th days. Their investigations thus show a good agreement with our quantitative chorionic gonadotrophin tests.

SUMMARY

(1) Up to December 1963 a total of 522 cases of combined extrauterine and intrauterine pregnancy were on record. The mother as well as both infants had survived the first month after delivery in only 11 of these cases.

(2) A new case is reported. The extrauterine pregnancy was diagnosed after the delivery of the first infant, prior to laparotomy. Both infants were delivered at term and were discharged with their mother on the 19th day after the operation.

(3) The excretion of hormones from the rather large abdominal placental remnant which was left behind was followed during the subsequent months. The excretion of oestriol was 0.5 mg/24 h. on the 10th day. The excretion of chorionic gonadotrophin was definitely raised (260 units) as late as the 39th day.

(4) On the basis of the literature it is concluded that the extrauterine placenta should not be removed unless the afferent vessels lend themselves to ligation easily.

early stage of pregnancy presumably because the rupture then occurs entirely unexpectedly outside hospital and in the absence of a doctor or midwife so that adequate treatment is delayed. Out of the 24 cases which up to 1954 had resulted in the delivery of live infants 4 mothers (16 6 per cent) died, but these deaths occurred before 1926 The *fœtal mortality* is far higher Among 435 combined pregnancies Vasicka and Grable found that only 43 of the intrauterine and only 13 of the extrauterine infants survived the neonatal period. This difference is due mainly to the smaller size and lower weight of the extrauterine infant. In the 12 cases in which both infants survived the neonatal period the difference in weight between the twins was slight, which indicates an adequate vascular supply to the ectopic twin. In our case the extrauterine infant weighed 400 g more than the intrauterine one Correspondingly the extrauterine placenta weighed at an estimate 200–300 g more than the intrauterine placenta

The Placental Remnant

Owing to thrombosis in the vessels in the placental remnant following ligation of the vessels between its two parts it must be assumed that a diffuse infarction of the placenta occurred. Following red infarction an advancing fibrosis, resulting in shrinkage of the placenta must be expected. From the very beginning, therefore we were on the look-out for possible clinical signs of toxæmia and also had our attention directed to the hormonal status after the operation

Clinically we found (Fig 6) an elevated blood pressure during the first 12 postoperative days, during a period where hæmatocrit determinations showed constant values. There was no albuminuria and the SGP transaminase was not at any time increased. Lactic dehydrogenase was not determined. The E.S.R. ranged from 20 to 30 and up to 36 mm/hour from the 1st to the 14th postoperative day whereupon it steadily decreased, reaching 15 mm/hour a little over 3 months after the operation. Thus, there is no definite aggravation of the slight preoperative toxaemia.

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THE DIAGNOSIS OF INTRA-UTERINE FŒTAL DEATH AND ELUCIDATION OF THE ÆTIOLOGY OF "MISSED ABORTION" BY MEANS OF SEMI-QUANTITATIVE GAS CHROMATOGRAPHIC DETERMINATION OF URINARY ŒSTRIOL AND PREGNANEDIOL

BY

LARS PHILIP BENGTSSON AND BJÖRN FORSGREN

Introduction

Intra-uterine fœtal death and retention of the dead fœtus present several problems of which the most important are

1. accurate diagnosis of fœtal death
2. ætiology of the retention of a dead fœtus
3. treatment of retention of a dead fœtus.

The present paper concerns the first two problems; the third is not discussed here.

Missed abortion

Retention of a dead fœtus is often called "missed abortion". This is an ill-defined and confusing term of which almost every author presents his own definition. At one extreme Fraenkel demands death of the fœtus before it is viable and retention of the dead fœtus to expected term or longer. At the other extreme, Istre (1957) applies the term to all cases where the fœtus is dead and the uterus does not exhibit effective contractions. As there is no agreement on the definition of "missed abortion" the term has been avoided as far as possible in the present paper

(5) Removal of the extrauterine placenta involves hysterectomy due to uncontrollable bleeding in about 60 per cent cases, but it means a shorter stay in hospital and a lower post operative morbidity.

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tained. This results in a progesterone dominated myometrium which cannot work effectively. At a later stage progesterone production also ceases, and in the absence of both hormones the myometrium cannot produce effective contractions. This theory is supported by a study of the urinary excretion of oestriol and pregnanediol in a series of 30 cases of recent foetal death and missed abortion (Bengtsson & Falk, 1964). It is also in accordance with Csapo's theories on the effect of oestrogens and progesterone on the myometrium (see reviews by Csapo 1959, 1961 and Bengtsson, 1962b) and Cassmer's (1959) finding that, after primary foetal death with a surviving placenta, the oestrogen production drops before the progesterone production.

An alternative theory presented by Csapo et al. (1963a, b) is based on the supposition that uterine activity during pregnancy is controlled by, among other factors, the relation between uterine volume (V) and placental progesterone (P): an increase of the ratio V/P starts uterine activity while stabilization of the ratio maintains pregnancy. Missed abortion is supposed to depend upon stabilization of the ratio V/P because both V and P decrease after foetal death. In a series of 23 cases of foetal death Csapo et al. demonstrated that an increase in V (produced by intra-amniotic injection of physiological saline) or a decrease in P (produced by intra-amniotic injection of hypertonic saline) induced effective uterine activity, rapidly resulting in abortion. Œstrogen was supposed to be present in sufficient amounts in all cases. The urinary excretion of oestriol and pregnanediol was not studied.

Bengtsson's theory has received support from Jung (1965), Csapo's from Jaffin et al. (1962).

In the present investigation we have attempted to further elucidate the aetiology of retention of a dead foetus, as well as to provide an aid to the diagnosis of this condition.

Material

Urinary samples from 90 cases of suspected foetal death were investigated. The samples were obtained from our department and

except to express the general concept of retention of a dead foetus

Diagnosis of foetal death

The clinical signs of intra-uterine foetal death are sometimes equivocal and it may take days or even weeks to obtain an accurate diagnosis. The clinician is provided with a very valuable aid in the determination of urinary oestriol, which shows a rapid and pronounced drop after foetal death, as first shown by Zondek (1954) and later by ten Berge (1960) Accurate methods of oestriol determination are however very laborious and time consuming

The present paper is concerned with the development of a simple technique for the semi-quantitative determination of urinary oestriol and pregnanediol suitable for application to the determination of foetal death The feasibility of using this method to obtain guidance on the duration of foetal death has also been investigated.

Ætiology of the retention of a dead foetus

Our knowledge of the factors which control myometrial activity in pregnancy and labour is very fragmentary (see Bengtsson, 1962 b) It is therefore not surprising that we do not know why the uterus sometimes refuses to expel a dead foetus for weeks, months or even years. Ever since the discovery of the effect of oestrogens and progesterone on the myometrial activity of laboratory animals vague ideas have been presented concerning the rôle of these hormones in the retention of a dead human foetus. It is only recently however that theories have been published based upon experiments in animals and women and upon analyses of endocrine conditions judged by urinary excretion of oestrogens and pregnanediol.

One theory (Bengtsson 1962 a) relates retention of the foetus to a temporal difference in the decline of the oestrogen and progesterone production following foetal death It is suggested that in the period immediately after foetal death oestrogen production falls rapidly whilst progesterone production is main-

tained. This results in a progesterone dominated myometrium which cannot work effectively. At a later stage progesterone production also ceases and in the absence of both hormones the myometrium cannot produce effective contractions. This theory is supported by a study of the urinary excretion of oestriol and pregnanediol in a series of 30 cases of recent foetal death and missed abortion (Bengtsson & Falk, 1964). It is also in accordance with Csapo's theories on the effect of oestrogens and progesterone on the myometrium (see reviews by Csapo 1959, 1961 and Bengtsson, 1962 b) and Cassmer's (1959) finding that, after primary foetal death with a surviving placenta, the oestrogen production drops before the progesterone production.

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alive at the time of investigation and that the pregnancy continued for at least one more week. "Spontaneous abortion" means that signs of threatened abortion were present at the time of urinary collection and a living foetus was aborted within a few days.

The age distribution of the cases of foetal death is presented in Table II. This distribution does not differ significantly from that of normal pregnancies at our department.

One of the purposes of this investigation was to study the hormonal excretion at different times after foetal death. It was, therefore, important to know the date of foetal death as accurately as possible. As is well known this is often difficult and sometimes impossible. In the present study we have calculated the date of foetal death in the following way In 32 cases the calculation was based upon disappearance of foetal heart beats and/or foetal movements In the remaining 26 cases the calculation was based upon other signs such as disappearance of pregnancy symptoms, comparison of the length of amenorrhea with uterine and foetal size, and the condition of the dead foetus after delivery The inaccuracy of this calculation necessarily leads to some overlapping of the two groups foetus dead less than four weeks and foetus dead more than four weeks Table III shows the gestational month at foetal death in the 32 cases in which the time of foetal death was known with reasonable accuracy It is naturally the case that when foetal death occurs late in pregnancy the exact date is more accurately determined than when it occurs earlier

Methods

The urine was collected over 24 h. No preservative was added. The samples were analysed within 48 h. or kept frozen at −20 C until analysis was convenient. One aliquot was taken for oestriol determination and another for pregnanediol determination. The chemicals used were of "pro analysi" or puriss. quality purified as recommended by Bush (1961) and Neher (1964) The initial steps of the determinations described below (hydrolysis and first extraction) are in accordance with the methods described by Brown (1955) and Klopper *et al* (1955)

Table I. *The Final Outcome of 90 Cases of Suspected Fœtal Death*

Normal pregnancy	4
Fœtus dead less than 4 weeks	[illegible]4
Fœtus dead more than 4 weeks	34
Spontaneous abortion	3
Hydatidiform mole	
Extra-uterine pregnancy	1
Not pregnant	2
	90

Table II. *Age Distribution of Cases of Fœtal Death*

Age in Years	Number of Cases
< [illegible]0	
20–29	[illegible]
30–39	[illegible]3
40–49	6
	[illegible]

Table III. *Length of Gestation in Cases with Known Date of Fœtal Death*

Month of Gestation	Number of Cases
5	3
6	5
7	
8	7
9	0
10	5
	3[illegible]

from several other obstetrical and gynaecological departments in Sweden. Brief clinical data were enclosed. A "laboratory diagnosis" was based upon the semi-quantitative determination of oestriol and pregnanediol. Weeks or months later we received notes on the final outcome of the cases, which is shown in Table I. In this table "normal pregnancy" indicates that the fœtus was

alive at the time of investigation and that the pregnancy continued for at least one more week. "Spontaneous abortion" means that signs of threatened abortion were present at the time of urinary collection and a living foetus was aborted within a few days.

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Semi-quantitative determination of oestriol

200 ml of the 24 h urine sample was heated to boiling under a reflux condenser. 30 ml of concentrated hydrochloric acid was then added through the condenser and boiling was continued for 30 min. After cooling, the urine was extracted once with 100 ml and twice with 50 ml ether. The ether extract was washed twice with saturated $NaHCO_3$ and then extracted twice with 50 ml N NaOH to obtain the phenolic substances. This phase was neutralized with 4 N HCl to pH 6–7 and extracted three times with 50 ml ether. The ether extract was dried with anhydrous sodium sulphate, filtered and evaporated under reduced pressure at 40 C. The dry residue was transferred with a few ml absolute ethanol to a glass stoppered test tube for acetylation. This was performed for 30 min at 70 C with 0.5 ml pyridine and 2.5 ml acetic anhydride. After evaporation of the reagents the dry residue was dissolved in 0.1 ml ethylene dichloride. One to two μl of this solution was taken for gas chromatography.

Semi-quantitative determination of pregnanediol

50 ml of the 24 h sample was diluted to 100 ml with distilled water. Twenty ml toluene was added, and the mixture heated to boiling under a reflux condenser. Concentrated hydrochloric acid (10 ml) was added through the condenser and boiling was continued for 10 min. After cooling the two phases were separated and the urine extracted twice with 20 ml toluene. The combined toluene extracts were washed with 2 × 25 ml N NaOH and then with 3 × 25 ml distilled water. The toluene extract was dried with anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure at 40 C. The dry residue was transferred to a glass stoppered test tube with a few ml absolute ethanol. The alcoholic solution was evaporated, and the dry extract acetylated for 30 min at 50 C with 0.5 ml pyridine and 2.5 ml acetic anhydride. After evaporation of the reagents the dry residue was dissolved in 0.1 ml benzene-absolute ethanol, 1:1, and a suitable volume, usually 1–2 μl, was taken for gas chromatography.

Gas chromatography

The gas chromatographic analyses were performed with a Perkin-Elmer Model 800 Gas Chromatograph equipped with a flame ionization detector The columns used were 2 m×2.2 mm internal diameter stainless steel tubes packed with 3 % SE 30 on Gas Chrom Z, 100–120 mesh. Nitrogen was used as the carrier gas. The temperature of the columns was varied between 225 C and 240 C depending upon the age of the columns and their ability to separate the extracts. The injector temperature was maintained at 300 C. The detector temperature was about 10 C lower than the column temperature. The carrier gas flow was varied between 40 and 50 ml per min. for the same reasons as for the column temperature variations The carrier gas velocity was measured at the outlet of the column at room temperature. The chromatograms were recorded with a Recti/Riter (Texas Instruments Inc.) giving 1 mA full scale readings, and the sensitivity of the detector was usually set at ×20 The retention times of acetylated oestriol and pregnanediol respectively were determined with pure substances before and after each analysis.

The method described permits semi-quantitative determination of urinary oestriol and pregnanediol in 4–6 hours, enabling one technician to run about ten samples per day

Results

In order to simplify the terminology acetylated oestriol and pregnanediol are referred to in this and subsequent sections as oestriol and pregnanediol.

In the gas chromatograms obtained by this technique pregnanediol appears after a series of peaks representing among other substances *17-KS* (Fig. 1) In the proliferati e phase of the menstrual cycle the pregnanediol peak is considerably lower than the other peaks in the secretory phase it equals but never surpasses these peaks. The oestriol excretion during the menstrual cycle is too low to produce a peak in the gas chromatograms obtained by this method.

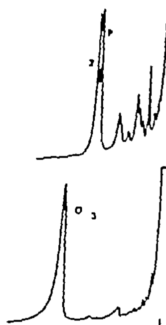


Fig. 1 Gas chromatogram of pregnanediol (above) and oestriol (below) in the 30th week of a *normal pregnancy* P pregnanediol, Oe oestriol. The figure \ to the left of the pregnanediol peak indicates that the true peak is twice as high.

In order to state the normal excretion pattern in pregnancy over 50 normal pregnancies have been studied at different stages by the method presented. One case has been followed by weekly determinations from the eighth week until after delivery Before the 13th to 15th week of gestation neither oestriol nor pregnanediol excretion differs significantly from that during the secretory phase From then on there is a steady and very pronounced rise in both oestriol and pregnanediol excretion throughout pregnancy Fig 1 illustrates the excretion pattern in the 30th week

In this study the oestriol peak is considered as "low when markedly lower than in normal pregnancies of the same gestational age and very low when the peak is hardly visible in the chromatogram. The relation between the pregnanediol peak and the other peaks (including 17-KS) in the cases under study and in normal pregnancies of the same gestational age has been com

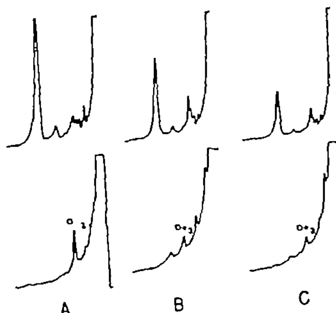


Fig. 2. Gas chromatogram of pregnanediol (above) and oestriol (below) after foetal death in the 30th week of gestation. Symbols as in Fig. 1. A *four days*, B *three weeks* and C *four weeks after foetal death*. (Œstriol appears earlier than in the other chromatograms due to higher column temperature.)

pared. On this basis pregnanediol excretion is considered as "low" when the pregnanediol peak is considerably lower than in normal pregnancy but well above secretory phase values, and "very low" when the peak is equal to or lower than in the secretory phase. Representative examples of normal, low and very low oestriol excretion are shown in Figs. 1, 3 A and 3 B respectively, and of normal, low and very low pregnanediol excretion in Figs. 1, 2 C and 6 respectively.

It is well known that the oestriol excretion drops rapidly after foetal death (Zondek, 1954, ten Berge, 1960, Cassmer 1959). It is not known, however, when pregnanediol excretion starts to drop and what values pregnanediol and oestriol may finally reach. In a few cases we have been able to follow the drop in oestriol and pregnanediol excretion by means of repeated

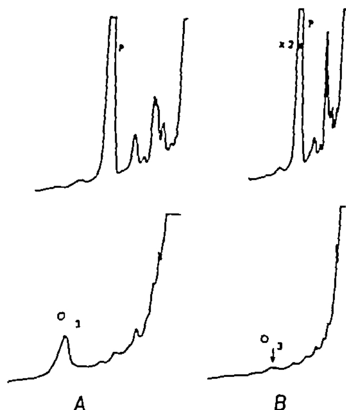


Fig 3. Gas chromatogram of pregnanediol (above) and oestriol (below) after foetal death in the 31st week. Symbols as in Fig. 1 *A fou days* and B *one month after foetal death.*

gas chromatographic determinations. In one case the hormonal excretion was studied by semi-quantitative determinations every second day from four days after foetal death to shortly before abortion one month later The history of this case is as follows

S.E 28 years old gravida IV with two early abortions and one spontaneous delivery at term. This pregnancy was normal up to the 30th week, when foetal movements and foetal heart beats disappeared. Admitted to our department four days after foetal death. Blood group A, Rh positive No albumen or sugar in the urine. Blood pressure 110/80 mm Hg Fibrinogen normal. Histaminase normal. The size of the uterus corresponded to the gestational age. The patient did not object to waiting for spontaneous deli ery so no treatment was given. Semi-quantitative determinations of oestriol and pregnanediol were performed every second day She was delivered spontaneously



Fig 4 Gas chromatogram of pregnanediol (above) and oestriol (below) five days after foetal death in the 33rd week. Symbols as in Fig

exactly one month after foetal death. The foetus showed third degree maceration length 3 cm, weight 790 g (with placenta) It exhibited multiple malformations (omphalocele kypho-scoliosis, abnormally short umbilical cord) The placenta showed pronounced atrophy

Four days after foetal death the oestriol excretion in this case was severely depressed but the pregnanediol excretion was normal (Fig. 2 A, cfr Fig 1) Three weeks after foetal death the pregnanediol excretion was still well above secretory phase values while the oestriol peak was almost undetectable (Fig 2 B) Four weeks after foetal death the pregnanediol peak was still somewhat elevated (Fig C)

In 16 cases of foetal death the urinary excretion of oestriol and pregnanediol was investigated 2–4 times after foetal death. These cases, as well as the single determinations in cases where the date of foetal death was known exactly showed the same pattern a rapid fall in oestriol excretion and a very slow fall in pregnanediol excretion. This is illustrated by representative examples in Figs. 3–6



Fig. 5. Gas chromatogram of pregnanediol (above) and oestriol (below) *13 days after foetal death* in the 36th week. Symbols as in Fig. 1

Table IV *Length of Gestation at Admission. Foetus Dead Less Than 4 Weeks*

Month of Gestation	Number of Cases
6	2
7	1
8	5
9	10
10	6
	24

The same pattern of decrease in oestriol and pregnanediol excretion is shown by the whole series of cases of foetal death. The cases have been divided into two groups, in one of which the foetus had been dead for less than four weeks and in the other for more than four weeks.

Tables IV and V show the duration of gestation at admission in the two groups Most of the cases in which the foetus had



Fig. 8 Gas chromatogram of pregnanediol (above) and oestriol (below) more than two months *after foetal death* in the 36th week. Symbols as in Fig.

Table V *Length of Gestation at Admission. Foetus Dead More Than 4 Weeks*

Month of Gestation	Number of Cases
4	3
5	5
6	
7	7
8	6
9	
	34

been dead for more than 4 weeks were admitted to the hospital before the 7th month, while the contrary was true of the cases in which the foetus had been dead for less than four weeks.

Tables VI and VII show the duration of foetal death in the two groups.

Table VI. *Duration of Fœtal Death. Fœtus Dead Less Than 4 Wee*

Duration in Week	Number of Cases
<1	13
1–2	4
2–3	4
3–4	3
	24

Table VII. *Duration of Fœtal Death. Fœtus Dead More Than 4 Wee*

Duration in Months	Number of Cases
1–2	6
2–3	1
3–4	1
Unknown but more than one month	26
	34

Table VIII. *Œstriol and Pregnanediol Excretion. Fœtus Dead Less Tha 4 Weeks*

Oestriol Excretion	Number of Cases	Pregnanediol Excretion	Number o Cases
Normal	1	Normal	18
Low	21	Low	6
Very low	2	Very low	0
	24		24

Table IX. *Œstriol and Pregnanediol Excretion. Fœtus Dead More Than 4 Weeks*

Oestriol Excretion	Number of Cases	Pregnanediol Excretion	Number of Cases
Normal	0	Normal	1
Low	8	Low	3
Very low	26	Very low	30
	34		34

Table X. Quantitative Determination of Œstriol Excretion in Five Cases of Foetal Death

Month of Gestation at Admission	Duration of Foetal Death	Oestriol Microg/24 h
7	1 week	500
9	2 weeks	280
8	month	380
7	2 months	< 00
5	>2 months	~20

Table VIII shows the excretion of oestriol and pregnanediol when the foetus had been dead less than four weeks. The oestriol excretion is consistently reduced but rarely reaches "very low" values. In most cases the pregnanediol excretion is normal. It is rarely "low" and in no case "very low". Typical chromatograms from this group are shown in Figs. 3 A, 4 and 5.

The hormonal excretion when the foetus had been dead for more than four weeks is shown in Table IX. Œstriol excretion now shows a further reduction and is usually "very low". The pregnanediol excretion is also "very low" in most cases. A typical example from this group is shown in Fig. 6.

There is thus an obvious difference between the two groups: when the foetus has been dead less than four weeks oestriol excretion is generally "low" and pregnanediol about normal; when the foetus has been dead for more than four weeks both oestriol and pregnanediol excretion reach "very low" values.

In the second group (foetus dead more than four weeks) the pregnanediol excretion often reaches values below those found in the secretory phase. In these cases the oestriol excretion is not large enough to produce a peak in the chromatogram. As it is of importance to know how far the oestriol excretion drops, we have in some cases determined the oestriol excretion quantitatively by other gas chromatographic methods (to be published by Forsgren and Nilsson). The results are presented in Table X, which shows that the oestriol excretion after long retention of a dead foetus may reach values corresponding to those of the menstrual cycle.

Table VI. *Duration of Foetal Death. Foetus Dead Less Than 4 Week*

Duration in Weeks	Number of Cases
<1	13
1–2	4
2–3	4
3–4	3
	24

Table VII *Duration of Foetal Death. Foetus Dead More Than 4 We*

Duration in Months	Number of Cases
1–2	6
2–3	1
3 4	1
Unknown but more than one month	26
	34

Table VIII. *Œstriol and Pregnanediol Excretion. Foetus Dead Less Th 4 Weeks*

Oestriol Excretion	Number of Cases	Pregnanediol Excretion	Number Cases
Normal	1	Normal	18
Low	21	Low	6
Very low	2	Very low	0
	24		24

Table IX. *Œstriol and Pregnanediol Excretion. Foetus Dead More Th 4 Weeks*

Oestriol Excretion	Number of Cases	Pregnanediol Excretion	Number Cases
Normal	0	Normal	1
Low	8	Low	3
Very low	26	Very low	30
	34		34

Table XIII. *Accuracy of Diagnosis Between Dead or Living Foetus. All Cases After the 15th Week of Gestation*

Dead Foetus		Living Foetus	
Dead Foetus		*Living Foetus*	
Correct	56	Correct	16
Dubious or incorrect	2	Dubious or incorrect	
	58		17

oestriol normal three days after clinical foetal death but low next day
oestriol depressed the foetus died soon after delivery
oestriol only slightly depressed, indicating a living (possibly endangered) foetus. Final outcome recently dead foetus.

E.A. 5th month of gestation. Pregnanediol normal, oestriol "low Our diagnosis: *recent foetal death*. Final outcome 13 days after determination delivery of living but weak foetus, which soon died.

R.S. 6th month of gestation. Pregnanediol "low oestriol normal. Our diagnosis: *living foetus*, probably an abnormal pregnancy. Final outcome premature delivery of living foetus.

M.T 7th month of gestation. Pregnanediol "low oestriol "low Our diagnosis: foetus dead more than four weeks. Final outcome hydatidiform mole.

R.O 5th month of gestation. Pregnanediol and oestriol normal. Our diagnosis. living foetus. Final outcome: abortion two days after determination condition of foetus unknown.

E.B 9th month of gestation. Pregnanediol normal, oestriol somewhat depressed. Our diagnosis: endangered foetus (?) Final outcome: recently dead foetus

It should be noted that in this series of 90 cases of suspected foetal death we found only one case with depressed pregnanediol but normal oestriol excretion (R.S. see above!) This case ended with premature delivery of a living foetus 11 weeks later

The only case of hydatidiform mole after the 15th week (M.T.) showed "low excretion of both oestriol and pregnanediol. (Four other cases of hydatidiform mole after the 15th week have been investigated. In all of them this diagnosis was suspected. They are not, therefore included in the material presented. All of them showed "low to very low oestriol ex

Table XI *Accuracy of Diagnosis Between Living Fœtus Fœtus Dead Less Than Four Weeks and Fœtus Dead More Than Four Weeks, All Cases Before the 15th Week of Gestation*

Correct	3
Dubious or incorrect	8
	11

Table XII. *Accuracy of Diagnosis Between Living Fœtus Fœtus Dead Less Than Four Weeks and Fœtus Dead More Than Four Weeks, All Cases After the 15th Week of Gestation*

Correct	72
Dubious or incorrect	7
	79

The results presented indicate that this semi-quantitative determination of urinary œstriol and pregnanediol permits a distinction to be made between

1 a living and a dead fœtus
2. a fœtus dead for less or more than four weeks respectively

As mentioned on p. 162 the excretion pattern of œstriol and pregnanediol during normal pregnancy observed by this method does not differ significantly from the secretory phase until the 13 th to 15th week. Consequently the diagnosis of fœtal death is unreliable before the 15th week of gestation (Table XI) After the 15th week when most missed abortions occur, an accurate differentiation between a living fœtus and a fœtus dead for less or more than four weeks respectively was made in 91 per cent of all cases studied (Table XII)

It should be noted that the demands for correct diagnosis in Table XII are rigorous as is evident from the following summary of the seven cases with "incorrect or dubious diagnosis

R.-M.B 6th month of gestation. Pregnanediol normal, œstriol "low Our diagnosis fœtus dead less than four weeks. Final outcome fœtus dead more than four weeks.

E.L. 10th month of gestation. Œstriol normal three days after clinical fœtal death, but "low next day

and progesterone production is one aetiological factor in the condition called missed abortion (Bengtsson, 1962 a)

This view on the aetiology of missed abortion is supported by Jung (1965) On the other hand, Csapo and his co-workers (1963 b) and Wood et al. (1962) do not favour the theory Csapo et al. (1963 b) found that the myometrium could be stimulated to contract effectively in all cases of foetal death also in a few cases of prolonged retention of the foetus. From this finding they drew the conclusion that there must always be a sufficient production of oestrogen. Unfortunately no oestriol determinations were performed. Intra-uterine instillation of hypertonic saline was capable of starting effective uterine activity in all cases presumably by depressing placental progesterone production, which was assumed to be at least partially maintained. No pregnanediol determinations were performed. Wood et al. (1962) found no relation between the length of time the foetus had been dead and the pregnanediol values. Of the 22 cases studied, however the foetus had been dead for as long as four weeks in only two cases and for five weeks in only one In our view the constitution of this sample readily explains the lack of relation between retention time and pregnanediol excretion as well as the rare occurrence of very low pregnanediol values

The difference in interpretation of results and in conception of aetiological factors may be due to the fact that both Csapo and his co-workers and Wood et al present cases in which retention of the dead foetus is generally of shorter duration than in the cases studied by Jung (1965) and ourselves.

It should be noted that the endocrine changes after intra-uterine foetal death are supposed to be only *one* of the aetiological factors in retention of a dead foetus There may well be other factors, such as the volume decrease as demonstrated by Csapo et al (1963 a b)

Methods of induction of uterine activity in cases of foetal death and missed abortion will not be discussed here. It should be mentioned, however that the possibility of differentiating between cases of foetal death of less or more than four weeks may be of importance in the choice of therapy (Bengtsson, 1962 a)

cretion and low" pregnanediol excretion. In these cases the hormone excretion pattern did not differ from that found in fœtal death.)

Discussion

The following discussion concerns only pregnancies later than the 15th week as the method used is not reliable in earlier stages.

In all cases of fœtal death a low œstriol excretion was found. The 13 cases with fœtal death one to six days before the investigation showed that the drop in œstriol excretion takes place immediately after the death of the fœtus. One case did not, however, show a fall until the fourth day after fœtal death. The sudden and rapidly progressing decrease in œstriol excretion, experimentally studied by Cassmer (1959) is thus a rather reliable indicator of fœtal death. Following this immediate rapid drop the œstriol excretion continues to decrease slowly down to very low values. In cases where the fœtus had been dead for two months or more the œstriol excretion was of the same order as during the menstrual cycle.

In contrast to the œstriol excretion, pregnanediol excretion drops very slowly after fœtal death so that up to four weeks after fœtal death normal or only slightly reduced excretion is found. The decrease is slow but continuous so that in cases of fœtal death exceeding four weeks normal pregnanediol excretion is rarely found. Finally, after fœtal death for two months or more the pregnanediol excretion approaches proliferative phase values.

In the present study a definite pattern of œstriol and pregnanediol excretion was found in all cases of intra-uterine fœtal death. It is tempting to assume that these specific endocrine changes, which have not been observed in any other condition, are at least partially responsible for the inactivity of the myometrium after intra-uterine fœtal death. The non-pregnant values of both œstriol and pregnanediol may imply that the production of œstrogen and progesterone is insufficient to enable effective contractions of the enlarged uterus. This study thus supports the assumption that the abnormal sequence of decrease in œstrogen

and progesterone production is one aetiological factor in the condition called missed abortion (Bengtsson, 1962 a)

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Methods of induction of uterine activity in cases of foetal death and missed abortion will not be discussed here. It should be mentioned, however that the possibility of differentiating between cases of foetal death of less or more than four weeks may be of importance in the choice of therapy (Bengtsson, 1962 a)

SUMMARY

A method is described for semi-quantitative gas chromatographic determination of urinary oestriol and pregnanediol in pregnancy. Ninety cases of suspected intra-uterine foetal death were investigated. In 58 cases the foetus was dead, in 24 for less than four weeks and in 34 for more than four weeks. Œstriol excretion falls immediately after foetal death and later reaches very low values. Pregnanediol excretion drops very slowly after foetal death and may remain normal for up to four weeks. Finally pregnanediol excretion also reaches very low values. The method is rapid and permits differentiation between a living foetus, a foetus dead for less than four weeks and a foetus dead for more than four weeks. The results indicate that specific endocrine changes are aetiological factors in "missed abortion".

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From the First Department of Obstetrics and Gynaecology (Professor A. Turunen) University Central Hospital Helsinki Finland

ε AMINOCAPROIC ACID (ε ACA) IN THREATENED ABORTION

BY

HARRY ZILLIACUS

Introduction

It is known that an enzymatic probably fibrinolytic process is involved in the mechanism whereby the trophoblast invades the decidua basalis and perforates the arterio-venous capillaries located beneath the epithelium. In many cases a slight physiological implantation bleeding occurs during this process. This bleeding may become profuse and pathological, when there is an imbalance between the bleeding and clotting mechanisms (Javert, 1957). Several investigators have shown that the endometrium and decidua are rich in substances promoting fibrinolysis (Albrechtsen 1956 Phillips Butler and Taylor 1956). Besides, strong fibrinolytic activity has been found in fœtal blood during early pregnancy (Zilliacus *et al* 1964).

The problem

Because fibrinolysis may play a role in the mechanism of abortion, it was thought that the administration of a substance inhibiting fibrinolysis might have a beneficial effect in cases of hæmorrhage in early pregnancy. With this in mind ε aminocaproic acid (ε ACA) known to be a potent antifibrinolytic drug (Okamoto *et al.* 1959) was given in 15 cases of bleeding in early pregnancy diagnosed as threatened abortion.

Results

ε-ACA in amounts varying from 6 to 30 g per day with an average of 13.6 g (total amount varying from 10 to 594 g, mean value 118.8 g) was *administered in 15 cases of threatened abortion* for periods of 1 to 30 days (mean 8.3 days). The ε-ACA was given when the pregnancy had lasted between 5 and 22 weeks. In one case the drug was administered in three periods, *i.e.* during the 17th, 19th–21st and 22nd week. (Table I.) In conjunction with the ε-ACA therapy progesterone and anti-spasmodics were administered in most cases. The bleeding remained unchanged in five cases. Although in seven cases the bleeding had stopped the day before ε-ACA was administered, the general impression was that there was probably a beneficial effect from the drug. Bleeding did not recur in these cases. In one case the bleeding stopped after 20 days' administration of ε-ACA.

As can be seen from Table I, there were three normal deliveries at term with live infants and one live premature delivery, the latter infant weighing 1930 g. Besides there was one premature delivery of an infant weighing 1130 g who died after three days because of prematurity. The other cases ended in abortion (one immature delivery, foetus 750 g).

Discussion

This investigation is to be regarded as a preliminary study on the feasibility of influencing possible pathological blood coagulation located in the area of placentation. In the mechanism of development of placental infarcts both haemostatic and bleeding processes are involved. That bleeding occurs from the foetal circulation into the mother's blood via the intervillous space is a well established fact. If local fibrinolysis plays a role, the use of fibrinolysis inhibitors may promote haemostasis in the affected placental area.

No teratogenic effect could be attributed to the use of ε-ACA in early pregnancy, since at least four cases resulted in the birth of normal live infants in this series.

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From the First Department of Obstetrics and Gynaecology (Professor A. Turunen) University Central Hospital Helsinki Finland

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BY

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The problem

Because fibrinolysis may play a role in the mechanism of abortion it was thought that the administration of a substance inhibiting fibrinolysis might have a beneficial effect in cases of hæmorrhage in early pregnancy With this in mind ε-aminocaproic acid (ε ACA) known to be a potent antifibrinolytic drug (Okamoto *et al* 1959) was given in 15 cases of bleeding in early pregnancy diagnosed as threatened abortion.

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Table I *Use of ε-ACA in 15 Cases of Threatened Abortion*

Total Bleeding in Days	ε-ACA		Administration of ε-ACA		Delivery or Abortion
	g/day	Total	Duration of Pregnancy in Weeks	in Days	
5	18	108	8	6	Abortion
3	30	150	15	5	Normal delivery 4010 g
25	20	504	6–10	30	Abortion
continuing	12	240	18	20	Normal delivery 3120 g
2	10	90	7	9	Abortion
continuing	15	180	18–19	12	Abortion
3	20	140	7	7	Abortion
4	6	18	14	3	Abortion
2	10	40	13	4	
3	10	50	15	5	Normal delivery 3060 g
2	10	50	5	5	Abortion
continuing	10	10	18	1	Immature delivery 750 g
8	10	70	21–22	7	Premature delivery 1930 g
continuing	10	50	20	5	Premature delivery 1130 g, died
continuing	25	550	17 19–21 22	22	Abortion
(Mean value)	(13.6 g)	(118.8 g)		(8.3 d)	

SUMMARY

ε ACA was administered in 15 cases of threatened abortion. The pregnancy terminated in abortion or immature delivery in 11 cases. Normal healthy infants were born in 4 cases (1 premature). The possible role of fibrinolysis in bleeding and hæmostatic processes in the placenta is discussed.

Table I.

Test	Normal Range
Whole Blood Clotting Time, glass	6– min.
plastic	15–25 min.
Bleeding Time (Duke)	1–5 min.
Thromboplastin Time (Quick 1945)	17–19 sec.
Factor II+VII (Owren and Aas 1951)	80–130 per cent
Factor V (Wolf 953)	80–120 per cent
Factor VIII (Nilsson et al. 1957)	60– 60 per cent
Platelets, $\times 10^3$	150–400/µl
Fibrinogen (Jacobsson 955, Nilsson and Olow 962)	0.2–0.4 gm/100 ml
Plasminogen (Amris 966)	70– 20 per cent
Plasma Thrombin Time (Amris 1966)	10– 2 sec.
Fibrinolytic Activity:	
Euglobulin Clot Lysis (Nilsson and Olow 962)	> 20 min.
Fibrin Plates (Astrup and Müllertz 952)	
Plasma	0-trace
Euglobulin	0–50 mm

trisodium citrate $2H_2O$ (1 part citrate + 9 parts of blood) The samples were centrifuged within 10–15 minutes after withdrawal and the plasma tested for fibrinolytic activity immediately after the centrifugation. The coagulation assays were performed on the fresh plasma or on plasma deep frozen immediately after centrifugation.

The methods used and the normal values of the different tests are given in Table I. The thrombin generation test was carried out by the method of Ollendorff (1960) and the thromboplastin screening test modified after Hicks and Pitney (1957)

Trasylol® (Bayer) vials of 5 ml 5.000 units per ml. Trasylol were administered intravenously either diluted in isotonic saline as an infusion or by slow intra venous injection.

Case Reports

Case no (journal B 824/65)

A 20 years old nulli-para. Estimated date of confinement was Aug. 7 965. Since the age of seven she had suffered from epilepsy treated with Diphydan, Mysoline and Zarodan. Her gynaecological and obstetrical history included an attack of salpingo-oophoritis in 96 and two uncomplicated abortions

Amris C. J. and Kjeldsen J. Acta obst. et gynec. scandinav. 45 180, 1966

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HÆMORRHAGIC DIATHESIS DUE TO ABRUPTIO PLACENTÆ TREATED WITH TRASYLOL[R]

BY

C. J. AMRIS AND J. KJELDSEN

Three theories have been advanced in explanation of the hemorrhagic diathesis associated with abruptio placentæ

1) Thromboplastic material passes into the maternal circulation causing intravascular coagulation and secondarily this gives rise to activation of the fibrinolytic system (Schneider, 1959)
2) Primary activation of the fibrinolytic system leading to hyperplasminæmia which causes the hæmorrhagic diathesis (among others Phillips *et al* 1962) and
3) Depletion of fibrinogen and clotting factors is largely related to the retroplacental coagulation process (Stouffer and Ashworth, 1958 Nielsen 1963) with deposition of large quantities of fibrin in the retroplacental hæmatoma.

The present report supports the theory that intravascular coagulation is the primary cause of the hæmorrhagic state in abruptio placentæ and that treatment with Trasylol a pharmaceutical which combines an antifibrinolytic and antithromboplastic effect, might be a new aspect in the treatment of the syndrome.

Materials and Methods

Blood sampling was carried out after separate venipunctures using ice-cooled non-activating plastic tubes with 3 8 per cent

placenta weighed 430 g and showed signs of separation of approximately 70 per cent of the surface. After delivery her condition improved steadily, blood pressure 10/70, and she had no abnormal bleeding. The hæmoglobin concentration was still low and the following day she had two blood transfusions. The post partum period was uncomplicated and she was discharged on the oth day after admission.

Case no 3 (Journal B.1080/65)

A 22 years old para-II with an estimated date of confinement of Aug. 22, 965. In 196 and 962 she had two normal deliveries. The present pregnancy had been normal. On June 3, 965, she woke up at 3.00 a.m. with lower abdominal pains and vaginal bleeding. She was immediately admitted to the local hospital. The patient was pale. The uterus was tense and tender with only slight revealed bleeding and the os admitted one finger. The heart sounds were varying 5–5 beats per minute. Because of a suspicion of abruptio placentæ grade II–III the patient was transferred to the Maternity Hospital.

On admission at 15.00 p.m. she was pale but warm and not sweating, blood pressure 00/70–90/70, pulse 20–30. The uterus was tense and tender especially on the right side. The foetal heart sounds were irregular and slow. The coagulation examination showed normal results and special treatment was not indicated. At 6.30 p.m. the condition was stable, blood pressure o/70, pulse 80–90 per minute. The foetal size was estimated to be less than 2000 g and the membranes were ruptured. The fluid was slightly hæmorrhagic. At this time no foetal heart sounds were audible. Further investigation of coagulation status gave satisfactory results and at 6.00 p.m. she delivered a stillborn boy of 800 g. Immediately after the placenta was borne together with 800 ml of partly clotted blood. The fundus contracted well and the post partum period was uncomplicated with no abnormal bleeding. The placenta weighed 570 g and showed signs of separation of approximately 1/4 of the surface and little white infarction. The patient was discharged on the 8th day after admission.

Case no 4 (Journal B 30/64)

A 7 years old nullipara with an estimated date of confinement of March 22, 964. Since Jan 3, 964, she had been treated with Dichloride (Centyl®) for slight oedema of the ankles and hands. Apart from this the pregnancy was normal.

On Jan 3, 964, in the 32nd week of pregnancy she was admitted t 6.35 m. For approximately 8 days she had felt no fœtal movements and shortly before the admission the membranes had ruptured. She was pale but in good condition. No heart sounds were audible and there was oozing of brownish malodorous amniotic fluid. At 9.00 a.m. Syntocinon® injections were started, and t 5 p.m. the patient delivered female anencephalic abortion of weight 320 g, crown-heel-length cm, but footlength 43 mm

in 1962 and 1963. The pregnancy had been normal until the actual episode which started on May 2, 1965 at midnight. During and after a ride in the switchback in an amusement park she developed diffuse abdominal pains, nausea and vomiting, but no revealed bleeding. The pains increased and at 9.00 a.m. the next day she was admitted.

On admission she was pale and sick with a blood pressure 130/90 decreasing to 90/-during the next hour. The uterus was tense and tender and no foetal heart sounds were audible. The blood failed to clot and oozing occurred from all puncture sites and petechiæ developed. At 9.30 a.m. the membranes were ruptured. The amniotic fluid was clear but soon became slightly hæmorrhagic. At 10.45 a.m. treatment with blood and Trasylol was started (see Fig. 1). Shortly after this the oozing stopped and her condition stabilized. At 2.15 p.m. she delivered a stillborn girl of 1550 g and immediately after the placenta was borne together with 2100 g of blood containing 455 g clots. The placenta weighed 330 g and 2/3 of the surface was impressed. At 2.20 p.m. the patient had a rigor just after the start of a new bottle of donor blood. After the transfusion was stopped and intravenous Antistina® was given the rigor ceased. At 2.40 p.m. she developed an attack of epileptic convulsions which were stopped immediately by intravenously administered barbiturates.

After the delivery no abnormal bleeding occurred and apart from the epileptic attack the post partum period was uncomplicated. The patient was discharged on the 13th day after admission.

Case no. 2 (Journal B 600/65)

A 21 years old para-II, gravida IV with an estimated date of confinement of April 27 1965. Her previous obstetrical history included two abortions 1962 and a normal delivery in 1964.

On the afternoon of March 27 1965 the patient noticed the uterus becoming hard and tender and she began to have constant lower abdominal pain, but no revealed bleeding. When she was admitted at 5.00 p.m. 1/2–1 hour after the start of the symptoms, the uterus was tense and diffusely tender and slight external bleeding was observed. No heart sounds were audible. She was shocked, the blood pressure falling from 100/70 to an undetectable level. The pulse was weak and thready 110–120 per minute. At 6.10 p.m. the membranes were ruptured. The amniotic fluid was slightly hæmorrhagic. The hæmoglobin was 7.0 g per 100 ml. The patient had oozing from all puncture sites and developed petechial bleeding beneath the blood pressure cuff. Examination of the coagulation status showed severe clotting defects and treatment with Trasylol and blood was started (see Fig. 1).

At 10.00 p.m. labour started, and at 10.15 p.m. she delivered a dead boy of 2400 g. Immediately after the placenta was borne followed by 1700 g of hæmorrhagic fluid +575 g of clots. After injection of Oxytocin (Syntocinon®) the uterus contracted well and no bleeding was observed. The

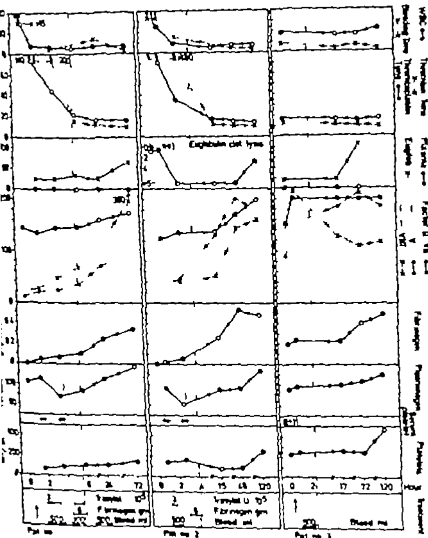


Fig. Changes in the coagulation status and treatment of 3 patients with abruptio placentæ. The vertical broken lines indicate the time of delivery. The arrows in the treatment diagrams indicate the time of rupturing the membranes.

the values are falsely lowered due to the action of Trasylol.

determined after addition of purified fibrinogen to the resuspended euglobulin.

corresponding to 24 weeks (Trolle, 1948). After delivery she began to bleed severely per vaginam and there was oozing from puncture sites. Coagulation examination revealed a severe hyperfibrinolysis. At 14.40 treatment with 200,000 units of Trasylol was started. Shortly after the Trasylol infusion was started the bleeding ceased and at 7.45 p.m. after additional treatment with fibrinogen 6 g (Table IV) her coagulation status had returned to normal. The placenta weighed 150 g and was without macroscopic abnormalities. Microscopy revealed residual decidua and many villi with a very fibrous stroma and fibrin deposits between the villi.

The post partum period was uncomplicated and the patient was discharged 8 days after admission.

Results

On admission patient no 1 had oozing from all puncture sites and developed petechial haemorrhages beneath the blood pressure cuff. Routine determination of whole blood clotting time and semiquantitative fibrinogen measurement indicated a fibrinogen level at zero. The special coagulation studies are shown in Fig. 1. It is seen that the clotting mechanism was severly impaired with very low concentrations of factor V, factor VIII, low fibrinogen, thrombocytopenia and prolonged bleeding time. The prothrombin-proconvertin (factor II+VII) concentration was within the normal limits and we could not demonstrate any increased fibrinolytic activity, abnormal decrease in plasminogen or decrease in plasmin inhibitor in serum. Addition of patient plasma to normal plasma only resulted in slight prolongation of the thrombin time, indicating few or no fibrinogen breakdown products in the patient's blood (Table II).

Phase contrast microscopy of the platelets revealed that the majority had abnormal morphology, like the "serum-platelets" described by Sokal (1962, 1963) (platelets changed after contact with thrombin—supposed to indicate a clotting process in or in direct relation to the circulating blood). Despite the severe clotting defects the patient's thrombin generation was accelerated (Fig. 2, solid curve). But the thromboplastin generation was retarded and decreased (Fig. 3). Addition of 20 per cent patient plasma to normal plasma, however, resulted in significant acceleration of the normal plasma's thromboplastin generation, indicating the presence of a clot accelerating substance in the patient's plasma.

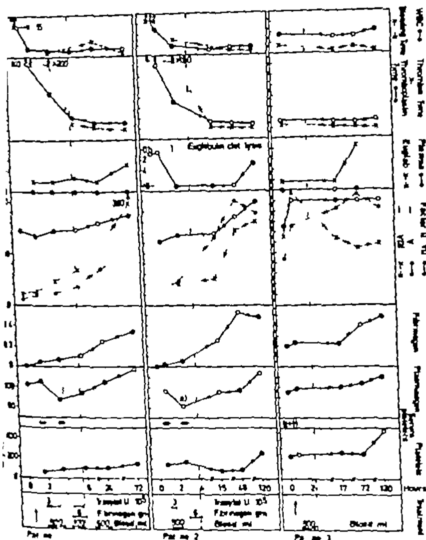


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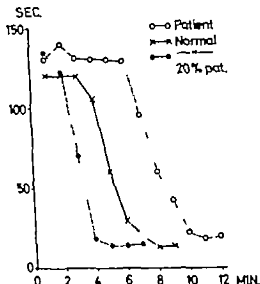


Fig. 3 The thromboplastin screening test of patient no. carried out before Trasylol treatment and demonstration of thromboplastic activator in the patient plasma

parameters increased to the values characteristic for the early post partum period.

Patient no. 2 was also bleeding and developed petechiae. She showed nearly the same coagulation pattern as did patient no. 1 (Fig. 1). However fibrinolytic activity was increased as judged from the short euglobulin clot lysis times. The euglobulin precipitate was incoagulable and the lysis times were determined after addition of bovine fibrinogen. Addition of patient plasma resulted in a moderate prolongation of the thrombin time (Table III). The platelet count was subnormal but in phase contrast microscopy the platelets were abnormal. The thrombin generation was of normal height despite the severe clotting defects. Just like patient no. 1 she had a retarded thromboplastin generation and admixture of the patient's plasma to normal plasma indicated the presence of a clot-accelerating substance in the patient's blood (Fig. 4). After treatment with Trasylol and blood

Table II. *The Antithrombin Effect of Plasma from Patient no. 1 Compared with the Effect of Normal Plasma*

Normal Plasma		200	200	200	200
Additional Plasma		50	100	150	200
Buffer		150	100	50	0
Thrombin 20 U/ml		200	200	200	200
Clotting	Patient	14	14	13.5	12
Time, sec.	Normal Control	12	10	10	9

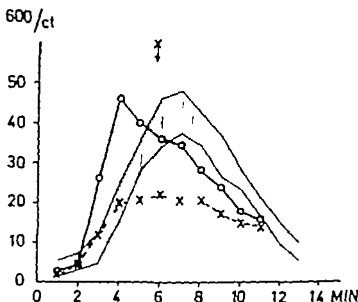


Fig. 2. The thrombin generation of patient no 1 before (o——o) and after (x - - - x) treatment with Trasylol. The shaded area indicates the normal range of the test (2xSD)

After rupture of the membranes was carried out (arrow in the treatment diagram Fig. 1) a very slight improvement in some of the coagulation parameters was seen. But the patient needed further treatment and Trasylol and transfusion with ordinary donor blood was started. During the treatment the bleeding soon stopped the coagulation status improved and the thrombin generation was retarded (Fig. 2 broken curve). The patient delivered a dead foetus without complications and without in creased bleeding. During the following days the coagulation

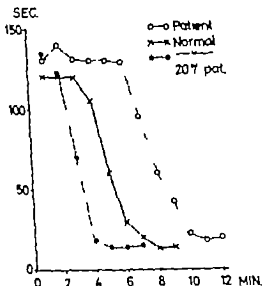


Fig 3 The thromboplastin screening test of patient no carried out before Trasylol treatment and demonstration of thromboplastic activator in the patient plasma

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Table III. *The Antithrombin Effect of Plasma from Patient no. 2 Compared with the Effect of Normal Plasma*

Normal plasma		00	200	~00	200
Additional plasma		50	100	150	~00
Buffer		150	100	50	0
Thrombin 10 U/ml		100	100	100	100
Clotting	Patient	24	23	21	18
Time sec.	Normal	17	16	14.5	13.5

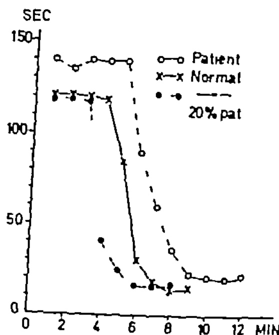


Fig. 4. The thromboplastin screening test of patient no. 2 carried out before Trasylol treatment and demonstration of thromboplastic activator in the patient's plasma.

the clotting time, thrombin time, bleeding time, fibrinogen and factor V improved slightly and the fibrinolytic activity ceased. After further treatment with fibrinogen she delivered without any complications. During the following days the coagulation parameters returned to values characteristic for the postpartal period with high concentrations of prothrombin-proconvertin, factor V and VIII and high fibrinogen concentration.

The platelet counts decreased temporarily after the delivery

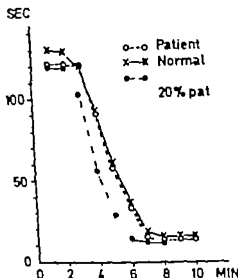


Fig. 5 The thromboplastin screening test of patient no. 3 and demonstration of a slight thromboplastic activator in the patient's plasma.

but no abnormal platelets were observed. The decrease may be due to disappearance of the abnormal platelets observed before the delivery.

Patient no. 3 had no bleeding symptoms. Fibrinogen was moderately lowered when compared with values characteristic for the stage of her pregnancy and the bleeding time was slightly prolonged (7 minutes) (Fig. 1). However all the other coagulation tests were within acceptable levels. Phase contrast microscopy revealed a few abnormal platelets. The thrombin generation test was normal and so was the thromboplastin generation (Fig. 5). In mixtures of normal and patient plasma a slight acceleration of the normal thromboplastin generation was observed. After rupture of the membranes factors II+VII and VIII increased significantly and after a single blood transfusion she delivered normally without any complication or bleeding. The fibrinogen concentration increased after the delivery to the characteristic high values.

Table IV *Changes in the Coagulation Status in Patient no. 4 after Treatment with 200 000 Units Trasylol and 6 g of Fibrinogen*

Test	Before Treatment	3 Hours After
Factor II+VII	125 per cent	135 per cent
Thromboplastin Time	34 sec.	18 sec.
Plasma Thrombin Time	>180 sec.	22.5 sec.
Whole Blood Clotting Time (Plastic)	>30 min.	14 min.
Platelets ×10	192/μl	185/μl
Thrombingeneration Test	normal	normal
Thromboplastin Screening Test	normal	normal
Fibrinogen	0.05 gm/100 ml	0.5 gm/100 ml
Whole Blood Clot Lysis	40 min.	>48 hours
Euglobulin Clot Lysis	<5 min.	>300 min.
Platelet Morphology	normal	normal

Patient no. 4 was brought to the department because of missed abortion. After the delivery she was oozing from all puncture sites and coagulation analyses showed very low fibrinogen, increased fibrinolytic activity and a prothrombin-proconvertin concentration corresponding to the stage of her pregnancy (Table IV). The platelets were normal and there was no demonstrable accelerator of clotting in the patient's plasma. We considered the bleeding as being a result of primary fibrinolysis and treated the patient with 200 000 units of Trasylol. Just after the start of the treatment the bleeding stopped and after infusion of fibrinogen the coagulation status was normalized.

Discussion

In only one of the patients with abruptio placentae was a moderate fibrinolysis demonstrated and the slight to moderate antithrombin activities measured indicated only few fibrinogen breakdown products in the patient's blood. The normal plasminogen values also are evidence against greater activation of the fibrinolytic system. The prothrombin-proconvertin concentration was within the normal limits in all the patients. However it must be remembered that prothrombin and especially procon-

vertin usually increases to high values (150–200 per cent) during pregnancy (Pechet and Alexander 1961)

Activation to thrombin of an amount of prothrombin equalling 5 10 per cent decrease may be sufficient for clotting of the total blood volume A prothrombin-proconvertin value within the normal range could therefore well indicate consumption in a pregnant woman.

The common findings in the patients with abruptio placentæ were

1) decrease of factor V factor VIII and fibrinogen
2) decreased platelet count (in two of the patients) and serum-platelets in the blood, and
3) the presence of a coagulation accelerator in the patients plasma.

These findings support the theory that defibrination and hæmorrhagic diathesis is caused mainly by liberation of thromboplastic materials into the maternal circulation. During the hypercoagulability disseminated intravascular fibrin formations may develop which might be responsible for deterioration of the patients general condition. It also might be responsible for the renal failure seen in some cases. At autopsy thrombi in the kidneys and fibrin emboli in the lungs have been demonstrated (Anthone *et al.* 1960 Reisfield, 1959)

Fibrinolytic dissolution of the thrombi before and after death is probably the reason why this is not a common finding. The increased fibrinolytic activity observed in some cases of abruptio placentæ is no doubt secondary to the disturbed hæmostatic balance and must be considered as an expedient mechanism securing patency of the vessels.

The mechanism responsible for liberation of thromboplastic material is still obscure. Passage of serum from the clotted retroplacental hæmatoma and material from decidua and placenta seems probable. It is well known that fresh serum contains strong clotting accelerators and thrombin, and a thromboplastin has been prepared from retroplacental blood (Teger Nilsson, 1964) If the primary cause of the hæmostatic breakdown is hypercoagulability the correct treatment must be directed

Table IV Changes in the Coagulation Status in Patient no. 4 after Treatment with 200 000 Units Trasylol and 6 g of Fibrinogen

Test	Before Treatment	5 Hours After
Factor II+VII	125 per cent	135 per cent
Thromboplastin Time	34 sec.	18 sec.
Plasma Thrombin Time	>180 sec.	22.5 sec.
Whole Blood Clotting Time (Plastic)	>30 min.	14 min.
Platelets ×10	192/μl	185/μl
Thrombingeneration Test	normal	normal
Thromboplastin Screening Test	normal	normal
Fibrinogen	0.05 gm/100 ml	0.25 gm/100 ml
Whole Blood Clot Lysis	40 min.	>48 hours
Euglobulin Clot Lysis	<5 min.	>300 min.
Platelet Morphology	normal	normal

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against this. Selective inhibition of the fibrinolytic activity by the use of epsilonaminocaproic acid (EACA) may well stop *external* bleeding due to fibrinolytic degradation of fibrin deposits. But the inhibition does not break the vicious circle, the increased coagulability and may increase the risk of fibrin formation in vital organs as the kidneys. Thrombus formation in connection with EACA-treatment has been reported (Naeye, 1962). In the case report on a patient with abruptio placentæ treated with EACA Albrechtsen and Skjodt (1962) mentioned *this risk* and in a *later* report the authors stressed this opinion (Albrechtsen and Skjodt 1964).

Heparin has been used in the treatment of intravascular coagulation (von Francken *et al.* 1963, Verstraete *et al.* 1963). Administration of heparin to parturient women is in our opinion a complicated matter demanding extensive clinical and special laboratory control if bleeding due to excess heparinisation is to be avoided.

The proteolytic inhibitor Trasylol has been known for some years as an excellent fibrinolytic inhibitor (Marx *et al.*, 1959, Beck *et al.* 1963). In higher concentrations Trasylol also seems to possess antithromboplastic effect, as it inhibits the earlier stages of coagulation and diminishes the effect of preformed thromboplastin *in vitro* (Amris 1964). This effect also seems effective *in vivo* after infusion of Trasylol to patients with bleeding and suspected intravascular coagulation (Amris, 1966). Trasylol does not interfere with the thrombin-fibrinogen reaction as heparin does. It can therefore be used without special precautions. In the patients in question Trasylol had a good clinical effect. The patients delivered without complications and their coagulation status improved. In view of the patients' severely impaired hæmostasis which usually results in prolonged and heavy bleeding at delivery, we consider this a good result of the treatment. Trasylol probably inhibits the hypercoagulability and in addition an increased fibrinolytic activity is stopped. In cases of primary fibrinolysis *Trasylol therefore will also be effective*. Patient no. 4 is an example of this.

In patient no. 3 the hæmostatic changes were moderate, and after rupture of the membranes her coagulation status improved.

A slight improvement was also seen in patient no. 1 after rupture of the membranes. This could be due to lowering of the intrauterine pressure and thereby a decreased passage of retroplacental material to the maternal circulation. Early rupture of the membranes therefore seems to be of some value in these cases. This is in keeping with the old clinical experience as to the beneficial effect of rupturing the membranes in abruptio placentæ.

The clearing of Trasylol from the circulation is fast (Werle and Trautschold, 1961) and the doses necessary to inhibit the normal clotting are rather high (Amris, 1966). In cases of suspected intravascular coagulation, e.g. postoperative bleeding we therefore have used 300,000–500,000 units infused intravenously during 15–20 minutes followed by 100,000 units per hour. The length of treatment has varied from 1 to 4 hours. One patient, a male with severe bleeding suspected to be caused by intravascular coagulation after gastric resection, was treated for 18 hours with good clinical effect.

Despite the severe clotting defects in cases of defibrination syndrome the blood may still have maintained its ability to clot and to lyse fibrinogen. Infusion of fibrinogen or blood prior to inhibition of the abnormal coagulation and increased fibrinolytic activity could therefore result in a fresh outbreak of a "burned out" intravascular clotting, as demonstrated by Albrechtsen and Skjødt (1964). For the same reason we start Trasylol treatment as soon as possible, and we never administer fibrinogen before Trasylol treatment has been started.

The results presented here are preliminary and we continue to investigate the effect of Trasylol, especially the problems concerning the dosage. Final conclusions are not yet possible but we think Trasylol is a new aspect in the treatment of the hæmorrhagic diathesis associated with abruptio placentæ, intrauterine retention of a dead fœtus and possibly amniotic fluid embolism.

SUMMARY

Three patients with abruptio placentæ and one patient with missed abortion were followed by frequent coagulation studies. The results support the theory that the hæmorrhagic diathesis

associated with early separation of the placenta is primarily caused by intravascular coagulation due to passage of thromboplastic material from the retroplacental hæmatoma to the maternal circulation. In the case of missed abortion the clotting defect was considered a result of primary activation of the fibrinolytic system.

Trasylol® a pharmaceutical with a combined antifibrinolytic and antithromboplastic effect, had excellent clinical effect and improved the patients' severely impaired coagulation status. Final conclusions are not yet possible. However Trasylol seems to be a new aspect of great importance in the treatment of the hæmorrhagic diathesis associated with abruptio placentæ missed abortion and possibly amniotic fluid embolism.

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TRANSAMINASE ACTIVITY DURING ORAL CONTRACEPTIVE THERAPY

BY

ULF LARSSON-COHN

In some articles particularly from Sweden and Finland (Eisalo *et al* 1964 Palva and Mustala 1964 Tyler 1964 Larsson-Cohn 1965 Eisalo *et al* 1965 Knutsson *et al* 1965 Borglin, 1965 Stoll *et al* 1965) altered liver function tests have been reported in women using oral contraceptives. Some cases of jaundice have also been described (Cullberg *et al* 1965 Larsson-Cohn and Stenram, 1965 Stoll *et al.* 1965 Carlstrom *et al* 1965) On the other hand many authors (Linthorst, 1964 Rice-Wray 1964 Swaab 1964 Swyer and Little 1965) have found no signs of impaired liver function.

The serum transaminase activity has been measured in a number of patients attending the Gynæcological Department of the University Hospital, Uppsala between August 1964 and July 1965, for the supervision of oral contraceptive therapy This is a preliminary report.

Material and Method

The study concerns 699 women between the ages of 16 and 43 years. At every visit blood was drawn for estimation of glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) In most cases where markedly elevated values were found, the estimations were repeated after an interval. In

certain cases, determinations of serum bilirubin thymol turbidity and alkaline phosphatase were made. In women with persistently elevated values oral contraceptive therapy was generally discontinued for a period of time The transaminase determinations were made according to Ordell's modification of the method of Karmen and Wróblewski. A Beckman B spectrophotometer was used and the reagents were made by AB Kabi, Stockholm. When values of over 30 units were obtained, repeat determinations were made on the same blood sample Values above 40 units were considered raised.

The blood samples were generally drawn in the afternoons without regard to recent meals. The transaminase activity was determined in 160 women before they started taking contraceptive tablets. The SGOT and SGPT were determined in 1288 different blood samples. The women had used the contraceptives for a total of 3520 months The following drugs were used Anovlar® (Norethisteroneacetate 4 mg+Ethinyloestradiol 0.05 mg) Conluten® (Norethisteron 2 mg+Ethinyloestradiol -3-methylether /EO³ME/ 0.1 mg) Lyndiol® (Lynestrenol 5 mg+EO³ME 0.15 mg) Lyndiol mite® (Lynestrenol 2.5 mg+EO³ME 0.075 mg) and Enavid mite® (Norethynodrel 5 mg+EO³ME 0.1 mg)

The tablets were administered according to the recommendations of the manufacturers. Anovlar and Lyndiol mite were taken for 21 and 22 days out of 28. Conluten, Enavid mite and Lyndiol were all started on the fifth day of a cycle and were administered for 21 20 and 20 days respectively In thirty cases, therapy was changed from one drug to another

Results

The results are summarized in Table I Table II shows the maximum values of SGOT and SGPT that were observed with the different drugs as well as the mean of the raised values.

One woman who at her first visit had already used Conluten for 9 months, had a SGOT of 520 U and a SGPT of 725 U Twelve days later the SGOT was 42 U and the SGPT 145 U After another 14 days the values were 39 U and 62 U respective-

Larsson-Cohn U Acta obst et gynec scandinav 45 196 1966

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TRANSAMINASE ACTIVITY DURING ORAL CONTRACEPTIVE THERAPY

BY

ULF LARSSON-COHN

In some articles particularly from Sweden and Finland (Eisalo *et al* 1964 Palva and Mustala, 1964 Tyler, 1964 Larsson-Cohn 1965 Eisalo *et al* 1965 Knutsson *et al* 1965 Borglin, 1965 Stoll *et al* 1965) altered liver function tests have been reported in women using oral contraceptives. Some cases of jaundice have also been described (Cullberg *et al* 1965 Larsson-Cohn and Stenram 1965 Stoll *et al* 1965 Carlström *et al* 1965) On the other hand many authors (Linthorst 1964 Rice-Wray 1964 Swaab 1964 Swyer and Little 1965) have found no signs of impaired liver function.

The serum transaminase activity has been measured in a number of patients attending the Gynæcological Department of the University Hospital, Uppsala between August 1964 and July 1965, for the supervision of oral contraceptive therapy This is a preliminary report.

Material and Method

The study concerns 699 women between the ages of 16 and 43 years. At every visit blood was drawn for estimation of glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) In most cases where markedly elevated values were found the estimations were repeated after an interval. In

Table III. Frequency of *Patients with SGOT or SGPT Values Higher* Than 100 U

Drug	Number of Patients	Number of Patients with SGOT or SGPT Higher Than 100 U
Anovlar	187	12 = 6 %
Conluten	334	5 = 4 %
Lyndiol	105	18 = 17 %
Lyndiol mite	58	3 = 5 %

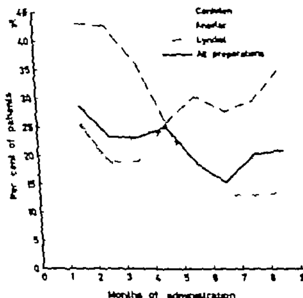


Fig. 1 Frequency of raised transaminases in relation to the duration of therapy

corresponding comparisons for SGOT because of the relative infrequency of elevated values.

Many of the patients with elevated transaminase values were tested again. Only the first of serial abnormal readings in any one patient was taken into consideration when the graphs in Figs. 1 and 2 were constructed.

Table I. *Frequency of Raised Transaminase Values with the Different Drugs*

Name of Drug	Number of Patients	Number of Cycles	Number of SGOT and SGPT Determinations	Number of Patients with Raised Values	Number of Patients with Raised SGOT	Number of Patients with Raised SGPT	Number of Pretreatment Determinations	Number of Elevated Values in Pretreatment Determinations	Number of Patients with Normal Pretreatment and Subsequent Elevated Values
Anovlar	187	1264	391	42=22 %	19=10 %	40=21 %	35	0	9=26 %
Conluten	334	1492	502	71=21 %	29= 9 %	67=20 %	33	2	12=36 %
Lyndiol	105	554	283	41=39 %	25=24 %	41=39 %	50	0	19=38 %
Lyndiol mite	58	138	62	13=22 %	3= 5 %	13=22 %	28	2	3=11 %
Enavid mite	15	72	50	1= 7 %	0	1= 7 %	14	0	1= 7 %
All drugs	699	3520	1288	168=24 %	76=11 %	162=23 %	160	4	44=27 %

Table II *Maximum Values and Mean of Elevated Values of SGOT and SGPT*

Drug	SGOT		SGPT	
	Max Value	Mean of Elevated Values	Max Value	Mean of Elevated Values
Anovlar	146	56	285	75
Conluten	520	78	725	93
Lyndiol	160	73	350	103
Lyndiol mite	122	66	360	85

ly The rapid fall in the transaminase activities suggests an acute disturbance but none was proved.

As the significance of only moderately elevated values is somewhat controversial Table III shows the number of patients in whom the SGOT and SGPT was higher than 100 U on any occasion As can be seen the incidence varied between 4 per cent and 17 per cent.

Fig. 1 relates the frequency of elevated SGPT values to the number of months of therapy Fig 2 shows the distribution of the raised SGPT values according to the day of the cycle on which the blood was taken It was not possible to make the

Table III. *Frequency of Patients with SGOT or SGPT Values Higher Than 00 U*

Drug	Number of Patients	Number of Patients with SGOT or SGPT Higher Than 100 U
Anovlar	187	2 = 6 %
Conluten	334	5 = 4 %
Lyndiol	105	8 = 17 %
Lyndiol mite	58	3 = 5 %

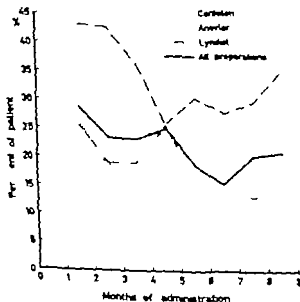


Fig. Frequency of raised transaminases in relation to the duration of therapy

corresponding comparisons for SGOT because of the relative infrequency of elevated values.

Many of the patients with elevated transaminase values were tested again. Only the first of serial abnormal readings in any one patient was taken into consideration when the graphs in Figs. 1 and 2 were constructed.

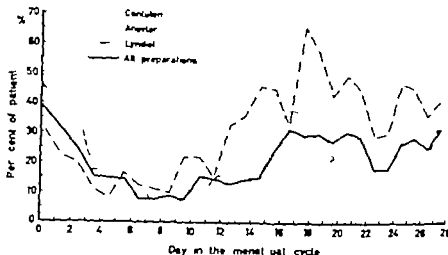


Fig. 2 Frequency of raised transaminases in relation to the day in the menstrual cycle when the blood was drawn.

Table IV *Frequency of Raised Values of Serum Bilirubin Thymol Turbidity and Alkaline Phosphatase*

Test	Number of Patients with Normal Values	Number of Patients with Raised Values
Bilirubin	101	1
Thymol turbidity	95	7
Alkaline phosphatase	100	2

Determinations of bilirubin, thymol turbidity and alkaline phosphatase were made in 102 patients with elevated transaminases One case of jaundice occurred in a patient using Anovlar This case was described elsewhere (Larsson-Cohn and Stenram 1965) Table IV shows the frequency of raised values

In some patients with persistently raised transaminase values treatment was discontinued for a time. If the transaminases fell to within normal limits, medication was recommenced. Table V shows the number of patients whose tests returned to normal while off drugs. Even those who retained raised transaminases showed a lower value than while on treatment

Table V *Transaminase Values in Patients with Raised Readings in Whom Treatment was Discontinued*

Drug	Number of Patients	Number of Patients with Normal Values after Discontinuing Treatment	Number of Days Without Treatment
Anovlar	19	16 = 84 %	4–56
Conluten	13	3 = 100 %	10–32
Lyndiol	21	18 = 86 %	0–49

In some cases elevated transaminase values reappeared when medication was resumed. However, the series is still too small to be presented. The same is the case with the patients in whom there was a change from one drug to another.

Discussion

In this series, the frequency of a rise in transaminase values was higher than that reported by most authors. However, Nilsson *et al.* (1965) from Gothenburg, Sweden, found that 5 of their 10 patients using Lyndiol showed elevated transaminase values. In the present series 39 per cent of the 105 women using the same drug showed abnormal values.

As can be seen in column 8 of Table I, 160 women were tested before they started taking the tablets. Later 27 per cent of them showed elevated values, a figure in keeping with the overall figure of 24 per cent for the whole series. Column 9 of Table I shows that 4 out of 160 patients had pathological values before they started taking the tablets. In none of these was the finding confirmed when the blood test was repeated and it may therefore have been due to laboratory error. If a similar incidence of errors is assumed throughout it would mean that 2–3 per cent of the transaminase readings were too high.

One woman was referred to the medical department because of a persistently raised transaminase activity and an elevated serum bilirubin. She was not given oral contraceptives.

Fig. 1 shows no convincing relation between the duration of

therapy and the incidence of raised transaminase values. The suggestion of a fall in frequency with duration of therapy may reflect the fact that some of the patients were taken off treatment at an early stage because of abnormal results. Fig. 2 shows that abnormal transaminase values occurred least frequently between the fifth and the eleventh days of a cycle. This suggests that there is a relationship between discontinuing tablets and a fall in transaminase with a lag of about one week.

From the figures and tables it can be seen that the frequency and level of pathological transaminase values was about the same in users of Anovlar, Conluten and Lyndiol mite but both levels were higher with Lyndiol. If the difference was merely due to the amount of gestagene in the various drugs than one would expect the patients on Enavid mite with its 5 mg of norethynodrel to show the same high incidence of raised transaminases. The small number of patients on this drug showed the contrary. It could be therefore that the lynestrenol in Lyndiol affects the liver more than the other gestagens.

It is interesting to note that most reports of altered liver function tests with contraceptive tablets have come from Finland and Sweden, countries which are believed to show an unusually high incidence of idiopathic jaundice of pregnancy (Ikonen, 1964). It seems unlikely that these facts can be explained on a nutritional basis for the diet in these countries is not markedly different from that in other communities with a comparable standard of living. It could be that some genetic deficiency in an enzyme system makes a part of the population in these areas prone to intolerance of situations demanding an increased metabolism of some steroids. In this context, it is perhaps relevant to note that several reports exist (Adlercreutz, 1964; Elliott and Hendry, 1965; Ikonen, 1964; Larsson-Cohn and Stenram, 1965; Holzbach and Sanders, 1965) of women who became icteric when taking oral contraceptives and had a previous history of idiopathic jaundice of pregnancy. The iatrogenic disease, which is caused by an intrahepatic biliary stasis is simillar in type to the disorder which occurs in pregnancy.

The Bromsulphthalein (BSP) retention test is thought to be

the most sensitive test of liver function and it has been shown that an increased BSP-retention is fairly common in women using oral contraceptives (Tyler 1964 Larsson-Cohn, 1965) The metabolism of the dye by the liver is very simillar to that of bilirubin (Goresky 1965) and can be divided into three phases, i.e. absorption, conjugation and excretion into the bile canaliculi. It is the excretion that is impaired by the steroids. This is in keeping with the histological picture of biliary stasis that is found in some of the cases.

A study is now in progress in which oral contraceptive therapy is being preceeded by a BSP retention test as modified by Castenfors and Hultman (1962) A dose of 20 mg BSP per kilogram of body weight is used. Blood samples are taken at 5 minutes intervals during the first half hour and then every 20 minutes until 2 hours have elapsed. A BSP retention of more than 3 per cent at 2 hours is considered pathological. The tests are being repeated after the tablets have been in use for 1 3 6 and 12 months and also one month after their withdrawal. Preliminary results in 20 patients show a raised BSP-retention in about 50 per cent.

SUMMARY

Determinations of SGOT and SGPT were made in 699 women who had used different oral contraceptives for a total of 3520 months. In all, 1288 different blood samples were analyzed and pathological values were found in 24 per cent of cases. In patients on Lyndiol there was 39 per cent incidence of raised transaminase values and the abnormal readings tended to be higher than in other groups Pathological transaminase values were found least frequently during the first part of the menstrual cycle and this may reflect a rapid response to drug withdrawal

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PRIMARY CARCINOMA OF BARTHOLIN S GLAND

Report on two cases

BY

E. PUROLA AND O. WIDHOLM

As a rule, primary carcinoma of Bartholin s gland is unilateral, although Lewis (1956) described a case in which squamous cell carcinoma of this gland was observed simultaneously on both sides. The condition is relatively rare. To our knowledge the only case described in Finland was published in 1943 (Laukola) Wharton and Everett mentioned that 109 cases had been reported by the end of 1951 and in a survey of the literature Masterton and Goss, in 1955, collected 116 cases Subsequently Barclay *et al.*, in 1964 found 27 cases in the literature and added 8 cases of their own and in a recent report Sackett (1965) described 3 new cases, so that the cases hitherto described number 154. Haines and Taylor (1962) however assumed that the true frequency of the tumour is not expressed by the number of case reports published, many advanced cases certainly being diagnosed as carcinoma of the vulva.

According to Simendiger (1939) the neoplasm is always clinically first observable as a small, firm, mobile non-tender tumour situated deep in the labium majus. As it gradually grows, it infiltrates the surrounding tissues including the skin of the labium majus, and at the same time becomes tender and liable to infection. Usually the patient seeks medical aid on account of pain, coital difficulties and secondary infection of the tumour

Since a neoplasm of Bartholin s gland may originate either in the duct or the acinus, both epidermoid carcinoma and adenocarcinoma occur the latter being more common. Papillary forma-

tions and secretion are sometimes encountered, a cribriform type of neoplasm is frequent. Willis (1953) emphasized that it is useless to attempt a strict classification by different types of growth since mixed forms frequently occur.

In regard to treatment the principles generally accepted in the treatment of carcinoma of the vulva have been recommended (TeLinde 1962). The best results have been obtained by vulvectomy followed either immediately or at a second stage by bilateral inguinal and femoral lymphadenectomy. Although the majority of authors have regarded the tumour as very resistant to irradiation, postoperative roentgenological treatment is generally regarded as desirable. The prognosis is poor since the patients mostly seek medical aid too late. The literature describes only a few 5 year survivors.

Case reports

Case 1 2/I/1964. The 65-year-old patient was the wife of an industrial worker. She had a history of rheumatoid fever and subsequent cardiac valvular disease. The menarche was at the age of 17. Menstruation had been regular and the menopause was at the age of 48. The patient had had six normal deliveries and no abortions or gynaecological infections. For some years she had noticed a tender spot in the vulva. During the year before she was seen a gradually growing tumour had appeared in this site and the tenderness had increased. On examination a firm, very tender tumour the size of a plum, was observed in the region of the left Bartholin's gland, and the skin was found to be reddened, but otherwise intact. The vagina showed senile atrophy, the uterus was relatively small and mobile, the adnexa could not be felt owing to obesity. By needle biopsy no specimen was obtained owing to the solidity of the tumour. An operation performed under local anaesthesia revealed that the tumour tissue was firm and the surroundings massively infiltrated, suggesting malignancy. A frozen section was prepared, which confirmed this suspicion. Since the patient was in very poor condition, suffering from hypertension and hear-failure, local extirpation of the tumour was performed as extensively as possible without lymphadenectomy. Postoperatively cytostatic medication (Sendoxan) and irradiation treatment were given. According to the pathologist, the removed tumour tissue was uniform. In dense hyaline connective tissue there were numerous islands of epithelial tissue varying in size, the smaller ones being partly solid. In the larger islands small apertures were observed in abundance. In these areas the structure of the tumour was reticular or cribriform. In the relatively few larger islands there were, amidst the epithelial tissue,



Fig. Case. In dense hyaline connective tissue there are numerous islands consisting of epithelial tissue and arranged as in cylindroma. (Hemat — van Gieson, ×40)

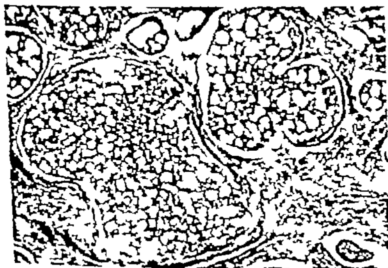


Fig. Case. The cribriform structure of the tumour tissue is clearly seen (Hemat — van Gieson, ×200)

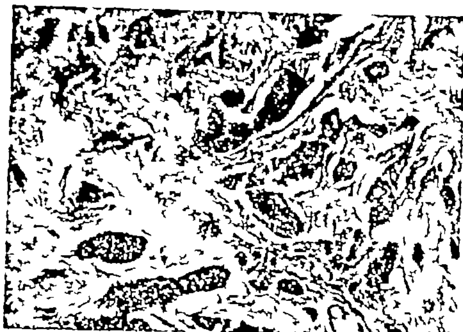


Fig. 3. Case 2. In dense connective tissue rich in cells, there are irregular solid strands and islands consisting of epithelial tumour tissue. (Hemat.—van Gieson ×40)

small rounded areas consisting of hyalinized connective tissue. A diagnosis of a malignant neoplasm originating in Bartholin's gland was made. It was unusual in structure, being in part cribriform, in part of the cylindroma type. The patient recovered well and was alive and without recurrence one and a half years after the operation.

Case 2. 334/I/1965. The 59-year-old patient was a mason's widow who had previously been in good health. The menarche was at the age of 14. Menstruation had been regular and ceased at the age of 52, when irregular bleedings sometimes occurred. The patient had had two normal deliveries and one abortion. During the first pregnancy in 1930, she had had bartholinitis, which recurred several times. Drainage had been performed twice, the last time in 1949. After this, Bartholin's gland had not been inflamed, but about one year before the patient sought medical aid, she had noticed in the vulva a small, non-tender, slowly growing tumour, which increased in size more rapidly during the two or three months before she was seen. There had been no pain or other symptoms, except slight vaginal discharge.

The examination revealed a nodose tumour, the size of a hen's egg, in the right labium majus. It was firm and fairly well defined, and appeared to extend as far as, but was not fixed to, the pubic bone. The tumour was not tender and the skin covering it was intact. The vaginal epithelium was

somewhat inflamed, cytological examination revealed senile colpitis. The uterus was normal in size and mobile. On needle biopsy small specimen was obtained, which was found to consist of infiltrating adenocarcinomatous tissue. Roentgenological investigation, cystoscopy and sigmoidoscopy revealed no signs of extension of the tumour Radical excision of the tumour vulvectomy and Basset s modification of inguinal and femoral lymphadenectomy were performed under general anaesthesia. Cytostatic medication was given during the first days after the operation. Later irradiation treatment was given.

On histological examination the tumour was found to be of epithelial origin. It grew in dense connectiv tissue rich in cells, forming mostly irregular solid strands and small islands. Here and ther in the tumour tissue there were larger areas showing highly typical glandular imitations. In addition, solid islands were present which exhibited cavities without any clear adenomatous structure. Throughout, the tumour consisted of rather small cells, poor in cytoplasm. The evidence was strongly in favour of adenocarcinoma originating in Bartholin gland. The recovery was normal and the patient was alive and had no recurrence six months after the operation.

Discussion

As has been emphasized by several authors (Novak and Novak 1958 Haines and Taylor 1962) it is often, particularly in advanced cases, difficult to decide with certainty from which tissue in the vulva a malignant tumour originates Honan (1897) expressed the opinion that a diagnosis of carcinoma of Bartholin s gland could not be regarded as established unless three of the following five criteria were satisfied 1) typical vulvar location, 2) position deep in the labium majus, 3) connection with the gland ducts, 4) the presence of intact glandular tissue 5) intactness of the skin over the growth. The two tumours described in the foregoing were clinically typical instances of primary carcinoma of Bartholin s gland. In addition, on microscopic examination one of them exhibited a very clear cribriform structure which is regarded as typical of carcinoma originating in Bartholin s gland (Barnes 1949 Hertig and Gore 1953) The other tumour was histologically less typical, but the assumption that it originated in Bartholin s gland is strongly supported by the clinical features and by the fact that the patient had had chronic bartholinitis twenty years previously

which may have contributed to the development of carcinoma. The rôle of infection in the aetiology of Bartholin's gland carcinoma is a matter of debate but the majority of authors, e.g. Harer (1933) have emphasized the importance of infection as a factor stimulating the growth of tumours.

SUMMARY

A survey of the literature is given, and two cases of adenocarcinoma originating in Bartholin's gland are described.

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CARCINOMA OF THE OVARY

A clinicopathological study of 86 autopsied cases with special reference to mode of spread

BY

FRANK BERGMAN

Numerically carcinoma of the ovary ranks third among female genital carcinomas and its mortality is still high, despite modern surgery and radiotherapy Only in comparatively early and low grade malignant ovarian neoplasms can a permanent cure be expected.

The dependence of prognosis on the histological type and degree of the tumour has received much attention. But so far no standard classification or nomenclature of ovarian tumours has emerged. This lack of a generally accepted classification often precludes comparisons of different series as well as of the value of different therapeutic methods used.

While many clinical reviews of ovarian carcinoma have been published (Lynch 1936 Meigs 1940 Pemberton, 1940 Heisel, 1946 Montgomery 1948 Allan and Hertig, 1949 Munnell and Taylor 1949 Taylor 1950 Hesseltine and Smith, 1956 Turner ReMine and Dockerty 1959 Purola, 1963 Gellé 1963 Gray and Barnes, 1962, 1964 Malloy Dockerty Welch and Hunt, 1965) much less emphasis has been placed on the variation in the spread of the disease with histological type of the tumour (Lay 1919 Janusz, 1926 Maljeff 1927 Kermauner 1932 Willis, 1941 Pearse-Behrman, 1954 Gellé 1963)

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This paper is concerned with postmortem analysis of 86 primary ovarian carcinomas of the germinal epithelial type classified in accordance with the recommendations of the Cancer Committee of the International Federation of Gynecology and Obstetrics (August 1961) Special attention has been given to the spread of the carcinomas as seen at operation and/or autopsy

Material and method of study

From July 1 1957 to June 30 1963 a total of 86 women with histologically proven primary ovarian carcinoma of the germinal epithelial type were autopsied at the Department of Pathology General Hospital Malmö This represented 1 2 per cent of all autopsies (6972) about 6 5 per cent of all female malignant tumours and about 36 per cent of all female genital malignant tumours seen during the 6 year period.

An attempt was made to classify the tumours according to the descriptions of their gross appearance in the operation records and autopsy reports. The primary tumours and the metastases were classified histologically by the examiner unaware of the case of which the specimens belonged. Occasionally there was a mixture of different cell types and then the diagnosis was based on the predominant type Only the primary tumours were graded, and only cases in which acceptable sections of the original neoplasm were available were accepted for the final study Cases of generalized abdominal carcinomatosis were accepted only when they were of proven ovarian origin. All microscopic slides were re-examined often repeatedly along with the patient s history and the operative findings.

The following organs were invariably examined histologically the ovaries uterus, lungs liver spleen kidneys and lymph nodes from the pelvis groins retroperitoneum (aortic) mediastinum, axillæ and supraclavicular fossæ (lymph nodes in supraclavicular fossæ examined routinely only in cases autopsied after January 1 1960—46 cases) All parts with gross evidence of metastases were also examined histologically The slides were stained with hæmatoxylin and eosin, besides which representative sections

were selected for further study with van Gieson's stain, McManus stain and mucicarmin. All together about 4500 slides were re-examined.

Results

The cases are grouped according to microscopic type and grade in Table I.

Table I. *Incidence of Histological Type and Grade of Malignancy of* 86 Consecutive Ovarian Carcinomas Seen at Autopsy

	Number of cases	Per cent of entire material
Serous cystomas		
) Serous papillary cystomas, benign		
b) Proliferating serous papillary cystadenoma without stromal invasion (possibly malignant) (Fig)	6	
) Serous cystadenocarcinoma		
I Low grade malignancy (Fig.)	27	
II High grade malignancy (Fig 2 b)	2	
	54	62 8
Mucinous cystomas		
) Mucinous cystadenoma, benign		
b) Proliferating mucinous cystadenoma without stromal invasion (possibly malignant) (Fig 3)		
) Mucinous cystadenocarcinoma		
I Low grade malignancy (Fig 4)	8	
II High grade malignancy		
	8	9 3
3 Endometrioid tumours		
)		
b) Proliferating endometrioid adenoma and cystadenoma (possibly malignant)		
) Endometrioid adenocarcinoma		
I Low grade malignancy (Fig 5)	4	
II High grade malignancy (Fig 5 b)	6	
	20	3 3
4 Undifferentiated carcinoma (Fig 6)	4	4 6
	86	00

This paper is concerned with postmortem analysis of 86 primary ovarian carcinomas of the germinal epithelial type classified in accordance with the recommendations of the Cancer Committee of the International Federation of Gynecology and Obstetrics (August 1961) Special attention has been given to the spread of the carcinomas as seen at operation and/or autopsy

Material and method of study

From July 1 1957 to June 30 1963 a total of 86 women with histologically proven primary ovarian carcinoma of the germinal epithelial type were autopsied at the Department of Pathology General Hospital Malmö This represented 1.2 per cent of all autopsies (6972) about 6 5 per cent of all female malignant tumours and about 36 per cent of all female genital malignant tumours seen during the 6-year period.

An attempt was made to classify the tumours according to the descriptions of their gross appearance in the operation records and autopsy reports. The primary tumours and the metastases were classified histologically by the examiner unaware of the case of which the specimens belonged. Occasionally there was a mixture of different cell types and then the diagnosis was based on the predominant type Only the primary tumours were graded, and only cases in which acceptable sections of the original neoplasm were available were accepted for the final study Cases of generalized abdominal carcinomatosis were accepted only when they were of proven ovarian origin. All microscopic slides were re-examined, often repeatedly along with the patient's history and the operative findings

The following organs were invariably examined histologically the ovaries, uterus lungs, liver spleen, kidneys and lymph nodes from the pelvis groins, retroperitoneum (aortic) mediastinum, axillæ and supraclavicular fossæ (lymph nodes in supraclavicular fossæ examined routinely only in cases autopsied after January 1 1960—46 cases) All parts with gross evidence of metastases were also examined histologically The slides were stained with hæmatoxylin and eosin, besides which representative sections

Fig. b.

Fig a–b Serous cystadenocarcinoma, a) low grade malignancy (cI) Gieson ×100 b) high grade malignancy (cII) Gieson ×100



Fig 3 Proliferating mucinous cystadenoma without [illegible]

Fig. 1 Proliferating serous papillary cystadenoma without stromal invasion (possibly malignant) (b) × Gleson ×100



Fig. 2

Fig. 5b

Fig. 5a-b Endometrioid adenocarcinoma, a) low grade malignancy (3cI) Gleson ×100. b) high grade malignancy (3cII) Gleson × oo

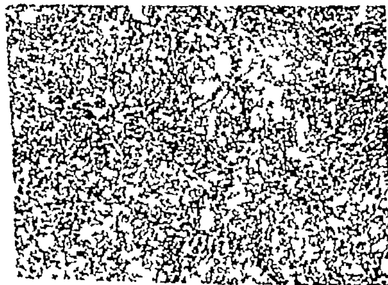


Fig. 6 Undifferentiated carcinoma (4) Gleson × oo

Fig. 4. Mucinous cystadenocarcinoma low grade malignancy (-d) v. Gieson ×100.

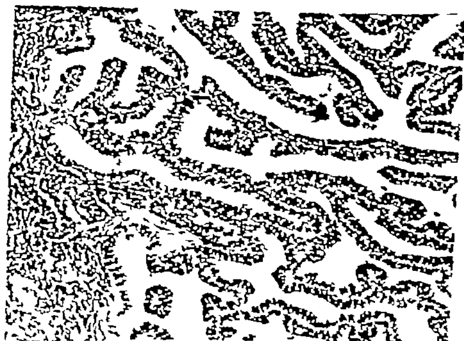


Fig. 5a.

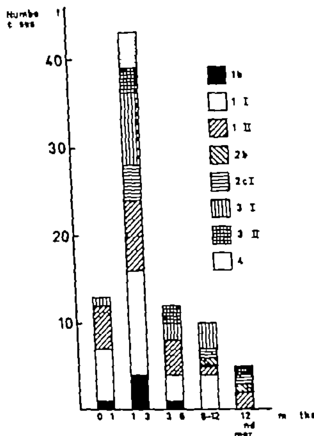


Fig. 6 Duration of symptoms from time of onset until diagnosis in 83 cases of ovarian carcinoma (In 3 cases not stated)

symptoms. At least half of the patients had abdominal pain or abdominal swelling due to the actual tumour or ascites. However ovarian carcinoma often produces no symptoms until it is well advanced. Examples of such an insidious onset of the disease were seen in three patients, aged 74, 76 and 91 in whom the tumours had produced no symptoms and were discovered at autopsy. Two of these patients had peritoneal carcinomatosis (with ascites in one case) and metastases in regional lymph nodes, but neither had remote metastases.

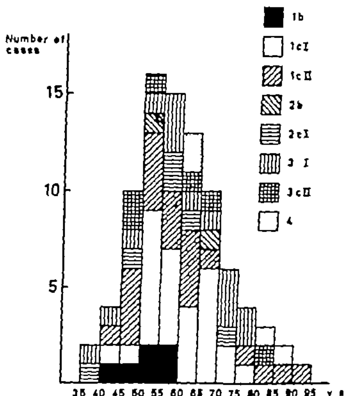


Fig. 7 Age distribution of 86 cases of ovarian carcinoma.

Primary classification and its relationships

Age The age distribution of the 86 subjects is given in Fig 7 Two-thirds of them were between 50 and 70 at the time of the diagnosis. The youngest patient operated upon for a mucinous cystadenocarcinoma, from which she died with widespread metastases ten months after operation, was 38 years old the oldest, 91

Marital status. Eighty per cent of the patients were married, widowed or divorced. Of the 69 married patients, 52 (75 4 per cent) had borne one or more children at term.

Almost eighty per cent of the patients were postmenopausal and no significant menstrual abnormalities were found among the premenopausal patients.

Symptoms. The list of symptoms was long and included some referable to the gastrointestinal urinary genital tract and other

Table III. Average Survival Time

Histological Group of Tumour	All Cancer Deaths		Untreated Cases		Treated Cases (Surgery and Subsequent Radiation)	
	No. of Cases	Survival in Months	No. of Cases Dead from Carcinoma	Survival in Months	No. of Cases Dead from Carcinoma	Survival in Months
b	5	20 9	1	(0.5)	4	43 5
1cI	26	4 5	5	2	12	22 3
cII	6	6.4	5	5 6	6	4 8
2b					0	–
2cI	5	32.2			3	(48.3)
3cI	2	8 6		(3)	5	29 0
3cII	6	8 7		(0)	2	(18 5)
4	4	2	2	(5)	0	–
	74	15 5	5	3 0	32	26.7

The deaths from cancer in each histological group are plotted as percentages of living patients after the diagnosis in Fig. 9 from which it is clear that by the end of the first year about 60 per cent of the patients had died, testifying the high malignancy of this type of genital cancer

Pathology

Bilateral tumours. The incidence of bilateral ovarian carcinomas found at operation and/or autopsy is shown in Table IV

Table IV Bilateral Tumours

Histological Group of Tumour	Number of Cases	Number of Cases with Bilat. Tumours	
			Per Cent
b	6	2	33 3
1	27	23	85
cII		5	7 4
2b			(50)
2cI	6	4	66 7
3cI	4	8	57
3cII	6	4	66 7
4	4	4	00
	86	6	70 8

The *duration of symptoms*, (Fig 8) *i.e* the interval between the onset of symptoms and the diagnosis was, on the average about 4 months but ranged from a few weeks to two years (a 72 year old patient with a mucinous cystadenocarcinoma, low grade malignancy) Fig 8 gives the duration of symptoms in the patients grouped according to the histological type of the tumours

Therapy More than half (48) of the 86 cases studied were inoperable The treatment given is apparent from Table II.

Table II *Type of Therapy*

Treatment	–	b	I	cII	b	3cI	3cI	3cII	4
None or exploratory laparotomy only	22	2	6	8	0	1	2	1	2
Radiation only	26	0	0	4	0	2	6	3	2
Operation only	1	0	0	1	0	0	0	0	0
Operation plus radiation	37	4	12	8	2	3	6	2	0
	86	6	27	21	2	6	14	6	4

Prognosis Of the 86 patients 74 had died from carcinoma, 4 postoperatively and 8 from intercurrent diseases Of the 2 patients with possibly malignant mucinous cystadenoma (group 2 b) one died postoperatively and the other 4 months after operation from pulmonary embolism

The average survival time after diagnosis, of the patients who died from carcinoma was 15 5 months Only 7.0 per cent survived more than five years after the diagnosis Those treated surgically with subsequent radiation therapy survived longest (average 26 7 months)

Of the untreated cases 68 2 per cent (15 cases) died from carcinoma on the average 3 months after the diagnosis (6 months after onset of symptoms)

In Table III the cases are grouped according to the average duration of survival after the diagnosis, histological type and grade of differentiation of the tumour

Table V Macroscopical Type of Tumour

Histological Group of Tumour	Gross Type of Tumour			
	Cystic	Semisolid	Solid	
b	3	3		
cI	1	9	7	
cII		3	6	
2b				
2cI		5	0	
3cI	2	8	4	
3cII		5	1	
4	0		3	
	1	54	21	Σ 86

Ascites. The incidence of ascites (over 200 ml) at autopsy was 61.6 per cent (53 cases). The amount of fluid varied considerably. It is noteworthy that the 53 cases were relatively equally distributed among the different histological types of tumours and almost all were associated with peritoneal carcinomatosis.

Extension of growth. The distribution of spread to various organs including the opposite ovary is given in Table 6 where no distinction is made between a single metastasis and extensi e metastases in a gi en organ or tissue.

It will be seen from the table that the tumour had spread beyond the ovary in 76 cases (88.4 per cent) Most of the initial involvement was by extension to the opposite ovary peritoneum—omentum and regional lymph nodes Of the 56 patients operated upon—in 18 cases only surgical exploration with *inter alia* omentectomy or biopsy of the omentum—metastasis to the omentum had occurred in 43 (76.8 per cent) by the time of operation.

However metastases were observed almost everywhere in the body The extent of the spread varied in the different histological groups, but the route of extension seems to be independent of the histological type of carcinoma and degree of histological malignancy

Myoma. The most commonly associated gynaecological disease was as expected, uterine leiomyoma, which occurred in 20

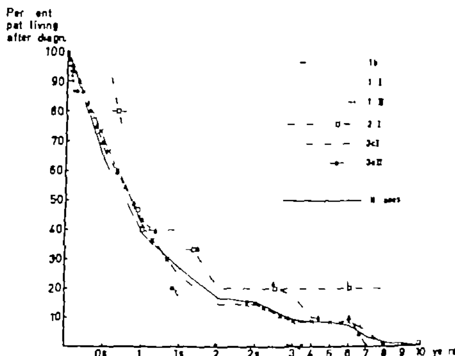


Fig. 9. Survival curves for 74 deaths in carcinoma of the ovary according to histological type and degree of differentiation.

70 9 per cent were bilateral The corresponding number found at operation of the cases treated was 60 5 per cent

Of the 25 unilateral carcinomas 17 (68 per cent) arose in the right ovary and 8 (32 per cent) in the left.

Gross appearance Information in the operation records and autopsy reports was not sufficient to classify the tumours according to size However it was clear that the vast majority of the mucinous tumours were large

An attempt was made to divide the material into cystic, partly cystic and solid growths (Table V) It is of course not possible to classify such tumours simply as solid or cystic because many that appear cystic have solid cores while many that appear solid are riddled with cysts

It is seen that a large percentage of the carcinomas were semi-solid and only the histologically possibly malignant tumours were grossly cystic The undifferentiated carcinomas were preponderantly of the solid variety

cases (23.3 per cent) Most of the myomata were small and of no clinical significance. Novak (1940) and Wharton (1943) gave the incidence of myoma as 20 and 26 per cent respectively in women over 30. Endometrial cysts and simple follicular cysts were occasionally found.

Multiple primary malignant lesions Six of the 86 cases had multiple primary malignant lesions. The remainder of the multiple primary foci—all extragenital—were in the breast (4 cases) kidney and bone marrow (myeloma) In 5 instances the additional neoplasm had existed prior to the discovery of the ovarian carcinoma, while one (carcinoma of kidney) was discovered at autopsy

Discussion

The incidence and grave outlook of carcinoma of the ovary more than merit the many publications which have appeared on this subject. However results obtained at different hospitals or by different therapeutic methods cannot be satisfactorily compared until a standardized histological classification has been accepted. A conference for this purpose was arranged in August 1961 by the Cancer Committee of the International Federation of Gynaecology and Obstetrics. On the basis of the variety of excellent classifications of ovarian neoplasms that emphasize the anatomic histogenic or endocrine features of these tumours (for review see Schiller 1940) the Committee introduced a classification for those ovarian neoplasms that are related to the germinal epithelium, because this variety includes most ovarian carcinomas This classification seems to be both clinically and pathologically practical and was used in the present autopsy study of 86 consecutive cases of ovarian carcinoma.

The material studied compared favourably regarding the incidence of ovarian carcinoma with that of other autopsy series (See Lubarsch and Henke 1937) It will be noted that the histological investigation at autopsy usually confirmed the primary histological grouping. As to the distribution of the tumours according to histological type no autopsy series was available for comparison. The papillary serous cystadenocarcinomas made

Table VI. *Location of Metastases Noted at Operation and Autopsy Number of Cases in Different Histological Groups*

Organ Involved	Histological Group							
	Total	b	I	tcII	b	xcI	ycI	ycII	4
Number of Cases	88	6	27		1	6	1	6	4
Opposite ovary	61	2	23	15	1	4	8	4	4
Uterus	16	–	7	5	–	1	2	–	1
Vagina	11	–	2	3	–	2	2	1	1
Vulva	1	–	–	–	–	–	–	1	–
Peritoneum	75	4	26	18	2	4	12	5	4
Omentum	61	1	23	16	2	3	10	4	2
L									
y Pelvic	69	4	24	16	–	4	12	6	3
m Aortic	67	3	23	17	–	4	11	6	3
p Mediastinal	43	4	18	8	–	3	6	4	–
h Supraclav left	23	–	8	5	–	2	3	3	2
n Supraclav right	21	2	7	5	–	1	2	3	1
o Axillary left	25	3	9	8	–	1	2	2	
d Axillary right	21	4	7	5	–	2	1	2	
e Inguinal left	37	3	16	9	–	3	1	3	2
s Inguinal right	31	1	14	9	–	1	1	3	2
Pleura left	25	2	12	6	–	–	4	1	–
Pleura right	32	3	15	5	–	3	4	2	–
Lungs	32	3	10	7	–	1	4	5	2
Liver	29	2	5	5	–	4	6	5	2
Kidneys	6	2	1	1	–	–	–	1	1
Adrenals	7	1	2	1	–	1	–	2	–
Brain		–	–	–	–	–	–	1	–
Pituitary	2	1	–	–	–	–	1		–
Bone	2	2		4	–		–	2	1
Spleen	5	–	–	1		2	1	–	1
Skin	7		2	1	–	1	1	1	–
Thyroid	2	–	–	–	–	1	–	–	1
Pancreas	1	–	–	–	–	1	–	–	
Heart		–	–	–	–	–	–		1

only cases (46) examined after Jan. 1 1960.

the diagnosis, and in most cases the carcinoma was relatively advanced when the patient was first seen at the hospital. The duration of symptoms is, of course, not an accurate measure of the duration of the disease, since the tumour must usually attain a moderate size before symptoms develop.

The attempt to correlate the histological type of carcinoma with the survival rate was only partly successful. The most favourable prognosis was found for the group of patients with the mucinous type of carcinoma. In these patients the duration of symptoms and survival in months after original diagnosis were longer than in those with the serous or endometrioid variety. Thus, although the total number of mucinous carcinomas was small in the present series, the results seem to substantiate the widely accepted view that these tumours are less malignant. There was however no detectable difference in survival rate between the patients with serous and endometrioid carcinomas.

When the survival rates were correlated with the grade of malignancy of the individual tumours a progressive decrease in survival was noted with increasing grade of malignancy (= poor differentiation). Hence, the prognosis seems to depend chiefly on the histological degree of malignancy and less on the histological type of the tumour. In clinicopathological studies of large series of ovarian carcinoma Long and Taylor (1964) Malloy *et al.* (1965) and Santesson (personal communication) found an appreciably longer duration of life in the endometrioid than in the serous group. These two findings—a better prognosis in a clinical series and an equally long history in an autopsy series—are not necessarily incompatible. It is possible that two diseases differ in death rate—and in prognosis in a clinical series—but that those who die as a result of the disease, and appear in the autopsy series die after an equally long time in both disease groups.

Dissemination of metastases was extensive. In the majority (88.4 per cent) extension had occurred by direct peritoneal involvement often with involvement of omentum but in some cases it was confined to the pelvis with infiltration into surrounding viscera. This is in agreement with the findings in other series (Riechelmann, 1902 Janusz, 1926 Maljeff 1927)

up more than half of the total series. Only a small number of carcinomas were of the mucinous type, but almost one fourth were endometrioid carcinomas Smith (1937) found about 22 per cent of the better differentiated ovarian carcinomas to be associated with, or related to endometriosis. In clinical series with primary carcinoma of the ovary Turner ReMine and Dockerty (1959) and Long and Taylor (1964) found the endometrial type of adenocarcinoma in 11 and 16 7 per cent, respectively

The clinical findings in this series agreed in general with those of other reports in the literature Some points however deserve comment. The mean age was about 60 years with the peak age incidence in the sixth and seventh decades, which agrees with Krasting's series (1906) but is about one decade higher than in most clinical series (Lynch, 1936 Pemberton 1940 Montgomery 1948 Allan and Hertig, 1949 Pearse and Behrman 1954 Turner ReMine and Dockerty 1959) On comparison of the various histological types no difference in this respect was found between the differentiated carcinomas, while the mean age of the patients with possibly malignant serous cystadenomas was almost 10 years lower and of those with undifferentiated carcinomas slightly more than 10 years higher than that of the patients with differentiated carcinomas.

Many authors have reported infertility to be common among patients with ovarian cancer ranging from 22 to 40 per cent (Lynch, 1936 Meigs, 1940 Pemberton, 1940 Dockerty 1945 Helsel 1946 Allan and Hertig, 1949 Munnell, 1952 Pearse and Behrman, 1954) The infertility rate in the present series, 24 6 per cent was lower than that in most reports, but seemed to support the view that patients with cancer of the ovary are less fertile as a group. The infertility did not, however appear to vary significantly with the histological type of carcinoma Neither was any relationship found between type of ovarian carcinoma and menopause or menstrual abnormalities.

The rapid course of the disease after the onset of symptoms was disappointing, in spite of the fact that this series by its very nature was essentially a study of the most unfavourable cases. Two thirds of the patients had symptoms for 3 months before



Fig. Embolic tumour material in lung capillary. Gieson × 400

in the patients who died after treatment and no detectable dif ference in this respect between the patients with different types of ovarian carcinoma.

Metastases to the myometrium, which were observed in 16 cases, may occur by extension through lymphatics (Taylor 1950) or by direct growth through the uterine wall. That lymphatics may also convey tumours from the ovary to the vagina and vulva is well known (Willis 1952). In the present series there was secondary carcinoma of the vagina in 11 cases (in one case also in the vulva). But in all these cases the pelvic peritoneum was involved with abundant tumour masses in the pouch of Douglas, from which the carcinoma could infiltrate the vagina.

The spleen was often massively lined externally with tumour masses which sometimes penetrated the capsule. In 5 cases, however, true splenic metastases were seen without involvement of the capsule. Pearse and Behrman (1954) mentioned 5 cases of splenic metastases in their series and in 1961 Yama-

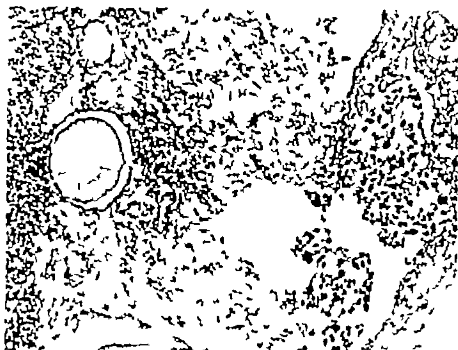


Fig 10. Metastasis in pituitary from an endometrioid ovarian carcinoma high grade malignancy v Gleson ×160.

In 1913 Schottländer found the peritoneum to be involved in 85 per cent of patients with metastasing ovarian carcinomas.

Approximately two thirds of the tumours were bilateral, which compares well with Montgomerys (1948) 70 per cent. Although difficult to prove at least in advanced cases with the pelvis almost completely occupied by tumour masses, it seems highly probable that most tumours arise in one ovary and spread to the other Such extension by lymphatics can sometimes be demonstrated. There was no apparent correlation between bilateral involvement and histological type of ovarian carcinoma.

Seventeen per cent (22 cases) of the total number of cases were untreated. Fifteen of this group died from carcinoma and hence can be assumed to have run the natural course of the disease These untreated patients lived, on the average 6 months after the onset of symptoms and all had peritoneal involvement and spread to regional lymph nodes as well as to the lungs and liver in 6 cases. There was a similar distribution of metastases

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guchi *et al* reported a case of splenic metastases in ovarian carcinoma that had been removed 3 years previously

Centrifugal lymphatic spread was common and occurred in the vast majority of cases to the regional lymph-nodes, but in some cases it produced almost generalized lymph vessel carcinomatosis.

Almost all cases with remote metastases, even of the pituitary (posterior lobe) (Fig 10) and thyroid showed not only almost bodywide spread to lymph nodes but also dissemination from the lymphatic system to the blood in the form of pulmonary metastases, sometimes only embolic tumour cells in the capillaries seen at histological examination (Fig 11) In only two cases, both with metastases in the vertebra there was no detectable pulmonary involvement and the osseous metastases may perhaps have been established by spread through perineural lymphatics

It would thus appear that ovarian carcinoma spreads primarily and predominantly by direct peritoneal involvement and lymphatic dissemination irrespective of the histological type and degree of the carcinoma. It is important that in this series no difference in this respect was found between the possibly malignant cystadenomas and the carcinomas

SUMMARY

The clinical and pathological data from autopsies on 86 women with primary ovarian carcinoma of the germinal epithelial type have been presented and the behaviour of the various histological types has been studied

In this series the histological type of tumour did not appreciably influence the duration of life and the only factor of prognostic value seemed to be the histological degree of malignancy

Extraovarian metastases were found in 88 4 per cent. Direct peritoneal involvement and spread by way of lymphatics were the predominant means of dissemination irrespective of the histological type and degree of the tumour

Table I. Frequency of *Vaginal Mycosis in a Series of 10,705 Patients*

...eries	Number of Cases Investigated	Yeast Culture Positive, Vaginal Smear		Yeast Culture Negative, Vaginal Smear Positive	Mycosis Detected	
		Positive	Negative		Number	Per Cent
... (Vaginal ...ly)	9,344	No yeast culture			473	4 6
... (Vaginal ...d culture)	334	26	88	4	118	35 3
... (Yeasts ... the vaginal	27	1		6	27	
	10,705	8 9	0	88		6

Methods

Culture method The specimens were taken initially into a liquid medium commonly used for the cultivation of *Trichomonas vaginalis* and yeasts (Penttinen et al 1948) and later transferred to Sabouraud medium. When pure cultures had been obtained, the yeasts were cultured on rice agar plates and studied for morphological characteristics. Fermentation and assimilation tests were performed on dextrose galactose saccharose, maltose and lactose In addition, a nitrate assimilation test was made All strains were cultured in Gorodkowa agar tubes which were examined for ascospores Lodder and Kreger-vanRij's taxonomy (1952) was followed.

Vaginal smears were obtained from the posterior vaginal fornix the ectocervix and the endocervix. Staining was performed by Papanicolaou's original method, by which fungi appear as bluish-red. The spore membrane takes up no stain, but it is readily distinguished from the environment by its strong refraction.

Results and discussion

Frequency As may be seen in Table I, in the group investigated both by culture and direct smear (series 2) mycosis was detected in 35.3 per cent. Vaginal smear alone gave a positive result in

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VAGINAL MYCOSIS

BY

S. TIMONEN O P SALO B. MEYER AND H HAAPOJA

In gynaecological practice vaginal mycosis is encountered almost daily Nonetheless the frequency of this condition has rarely been analysed. One of the most extensive surveys is Kimmig and Rieth's series of 2604 patients (1961) in which the frequency was 27 5 per cent In Finland Rauramo and Penttinen (1948) detected mycosis in 25 per cent of pregnant women and likewise in 25 per cent of patients with vaginal discharge Most authors have concluded that yeast occurs more often during pregnancy than in non-pregnant women (e g Baum 1958)

The purpose of the present study was to investigate the occurrence of yeasts in cultures from a series of gynaecological patients and to compare the results with those obtained from examination of vaginal smears.

Material

The material, which is presented in Table I consisted of specimens obtained from a total of 10 705 patients. The majority of the specimens i e 10 344 were vaginal smears taken routinely In 473 of these infection was detected This group called series 1 in what follows was subjected to further studies In addition, specimens from 334 outpatients chosen at random (series 2) were examined both by culture and by direct smear and 27 further cases (series 3) in which a vaginal smear had exhibited infection were also studied by culture

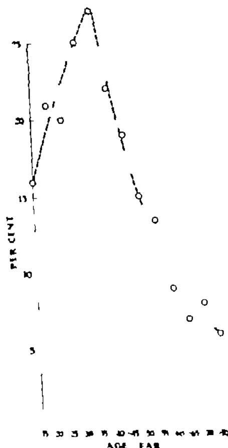


Fig. Relative age distribution of the patients with vaginal mycosis in series. The figures are calculated as percentages of random sample of 239 outpatients. The differences between age groups 26–35, 36–50 and >5 are highly significant.

only 4 6 per cent, culture alone in 34 per cent while the smear was positive and culture negative in 4 cases. It appears that only one in seven cases of mycosis can be diagnosed on the basis of a direct smear alone. Hence it is possible that those 4 6 per cent (series 1 Table I) who were detected among the 10 344 patients examined only by vaginal smear represent cases with heavy infection.

Table II *Age Distribution of Different Types of Vaginal Mycosis in Series 2 and 3*

Yeast Species	Age Group													Total Number	Per Cent
	≦ 5	6—20	—25	26—30	3 —35	36—40	4 —45	46—50	5 —55	56—60	6 —65	66—70	> 70		
Candida albicans		5	5	5	8	4	3	6	10	2	3	1	1	53	38 2
Candida krusei			2	1	2	1	1							7	5.0
Candida tropicalis		1												1	0 7
Candida parapsilosis						1	2	1				1		5	3.6
Candida reukaufii				3	1				1	1				6	4 3
Candida guilliermondi			1							1				2	1.4
Torulopsis glabrata		2	6	7	8	2	7	4	2	5		1	1	43	31.0
Torulopsis famata							1							1	0 7
Rhodotorula mucilaginosa						1	1		1		1			4	2 9
Rhodotorula pallida									1					1	0.7
Untyped				4	2	2	2	3	1	2				18	11 5

Table III. Œstrogen Effect of Vaginal Cells in Series 2 and 3

Œstrogen Effect	Yeast Species											Total Number of Cases
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Total number of cases	53	7		5	6	2	43		4		6	139
Low	4						3				1	1
Normal	32			2	4		25		1		7	76
High	4	3					3				3	3
Estimation not possible	13						1		3		5	39

against 42 per cent for the latter as calculated on the basis of the total number of cases with mycosis. In regard to other species no age tendency was discernible, but this may have been due to their rareness.

Endocrine aspects. An attempt was made to elucidate the possible relationship between mycosis and endocrine disturbances. Taking into account the time of the cycle and the age of the patient the œstrogen effect was evaluated as low, moderate or high in all vaginal smears from the patients with mycosis, so long as the infection did not prevent this evaluation (Tables III and IV). Among the cases diagnosed from cultures, low and high œstrogen values were equally frequent. The degree of œstrogen activity did not appear to influence the type of mycosis in this group of cases. By contrast, among the cases detected from vaginal smears alone, high œstrogen values were about twice as common as low values (Table IV). This seems to indicate firstly that there is no clear correlation between mycosis and hormonal disturbance (Table III) and secondly that mycosis leads to non-specific proliferation which is easily taken for an œstrogen effect (Table IV). The age distribution of mycosis justifies the conclusion, however, that the yeasts and Trichomonas grow best in the glycogen containing environment which is typical of child-bearing age. Previous authors have directed

The occurrence of different yeasts in the various age groups of series 2 and 3 is shown in Table II. The commonest species was *Candida albicans* which was found in 38.2 per cent of the cases with mycosis *Torulopsis glabrata* with a frequency of 31.0 per cent was the next commonest. Other species, of *Candida (C krusei C tropicalis C parapsilosis, C reukaufii* and *C guilliermondi)* taken together were found in 15.0 per cent of the cases. The frequency figures for *Candida albicans* and *Torulopsis glabrata* are in agreement with the results of previous investigators (Kimmig and Rieth 1961 Rauramo and Penttinen 1948 Thomsen Pedersen, 1960) Data regarding the occurrence of the less common yeasts have varied, or these species have not been detected at all.

Age distribution *The age distribution of mycosis* was plotted against the age distribution of a random sample of outpatients (Fig 1) A rather abrupt peak was observed at 31–35 years, although infection was found to be frequent also in the group 26–30 years Subsequently the relative frequency showed a steady decrease In their investigation of vaginal mycosis Petrů *et al* (1956) observed that *Candida* infection was most frequent in the age group 21–30 years while infection with *Torulopsis* was equally common in all age groups. Since they did not plot their results against the general age distribution of their patients, the significance of their figures is questionable, however The same objection is pertinent in regard to a study by Clark *et al.* (1959) which showed, however that the incidence of *Candida* infection reached its maximum at an earlier age as compared with infection with *Trichomonas*. When comparing the age distribution of 100 patients with candidiasis with the age distribution of a general population, Daftary *et al* (1963) detected no significant difference The difference observed in the present study between the age distribution of vaginal mycosis and the age distribution of a general gynaecological series is statistically significant

It appears that *Torulopsis glabrata* does not grow so well in patients over 50 years of age as does *Candida albicans* the frequency of the former being 22 per cent in the older age groups

Table V 6. Cases of Vaginal Mycosis Classified by Degree of Purity of the Specimen. 1–1 Represents "Normal" Variation. 2–3 Indicates Inflammatory Flora and Increased Number of Leukocytes (of Heurlin 1914)

	Yeast Species													
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Total by culture (Series 2 and 3)	Smear Only (Series 1)	Total Number of Cases
Number of Cases	53	7	1	5	6	2	43		4	1	6	139	473	6
Degree of purity 3	22	3		3			22		2		9	65	184	329
2		3			6		8		1		4	54	256	31
1				2			3				3	20	53	73
Cytolysis	9						8					8	63	81

Table VI. The Number of Leukocytes, the Frequency of Trichomonas vaginalis, Bacterial Flora and Papanicolaou Class in Series 1 and 3

	Yeast Species													
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Total by culture (Series 2 and 3)	Total by Vaginal smear (Series 1)	Total Number of Cases
Number of cases investigated	53	7		5	6		43		4		6	39	473	6
Leukocytes													36	36
Leukocytes +							22				7	43	50	93
Leukocytes + +	25	4		3		1					5	59	92	25
Leukocytes + + +	6				3		4		1		4	32	95	127
Trichomonas	4				1							8		20
Döderlein bacilli	8	6			4		3				8	8	365	446
Coccal flora				3			6		4		6	33	32	67
Mixed flora	4						8					23	–6	29
Papanicolaou class II	8										4	16		6
Papanicolaou class III–IV		1										5		5

Table IV. *Œstrogen Effect of Vaginal Cells in Series 1 (473 Cases of Vaginal Mycosis)*

Age Years		≦15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	>70	Total Number of Cases
Number of Cases		1	33	66	77	83	60	51	38	29	15	10	6	4	473
Œstrogen effect	Low		4	5	6	8	3	4	5				1		36
	Normal		9	38	46	40	27	26	14	16	2	5	2	1	226
	High		2	6	10	12	15	3	8	5	4	1	1		67
	Estimation not possible	1	18	17	15	23	15	18	11	8	9	4	2	3	144

Table V. 6 2 Cases of *Vaginal Mycosis Classified by "Degree of Purity" of the Specimen. 1–2 Represents "Normal" Variations; 2–3 Indicates Inflammatory Flora and Increased Number of Leukocytes (af Heurlin 1914)*

	Yeast Species													
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Unclassified	Total by culture (Series and 3)	Smear Only (Series)	Total Number of Cases
Number of Cases	53	7		5	6		43	1	4		6	39	473	6 2
Degree of purity	22	3		3		2	22				9	65	64	229
		3			6		18				4	54	56	3
3				2			3		1		3	20	53	73
Cytolysis	9						8					8	63	8

Table VI. *The Number of Leukocytes, the Frequency of Trichomonas vaginalis, Bacterial Flora and Papanicolaou Class in Series 1 and 3*

	Yeast Species													
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Unclassified	Total by culture (Series and 3)	Total by Vaginal smear (Series)	Total Number of Cases
Number of cases investigated Leukocytes	53	7		5	6		43	1	4		6	39	473	6
Leukocytes +													36	36
Leukocytes + +	25	4		3			22				7	45	50	98
Leukocytes + + +	16						7				5	59	9	29
Trichomonas	4				3		4				4	32	15	27
Döderlein bacilli	33	6										8		30
Coccoid flora	1				4		3				8	6	365	446
Mixed flora	5			3	2		6		4		6	35	32	67
Papanicolaou class II	8						8					23	76	99
Papanicolaou class III–IV											4	6		6
												5		5

attention to this circumstance as possibly explaining the frequency of mycosis during pregnancy

Pathogenicity The relationship between mycosis and other types of inflammation was studied by af Heurlins (1914) method of estimating the degree of purity At the same time the dominant microorganism was recorded As may be seen in Table V *Torulopsis glabrata* appears to grow in a purer environment than the remainder of fungi This finding is in agreement with the commonly accepted view (*e g* Kimmig, 1961 Petrů *et al* 1956) that *Torulopsis glabrata* is less pathogenic than *Candida albicans*

The same conclusion may be drawn from a study of Table VI which shows the relationship between mycosis and the number of leukocytes as classified by three degrees. As compared with *Torulopsis glabrata Candida albicans* was found to be associated with a greater degree of inflammation and Table VI shows that *Candida albicans* more often occurred in conjunction with *Trichomonas* In series 2 and 3 trichomoniasis was concurrent with mycosis in 8 cases (5 8 per cent) In series 1 in 12 cases (2.5 per cent) which makes a total of 20 cases and an incidence of about 3 3 per cent These figures are lower than the frequency of trichomoniasis (20 per cent) noted at our Outpatient Department (Timonen and Vartiainen 1961) The view that yeasts often occur in conjunction with *Trichomonas* is not corroborated by the present results On the contrary the opposite seems to be true

Furthermore the bacterial flora was correlated with the age of the patients (Table VII) It emerged that in the oldest age groups the incidence of mixed flora was relatively higher than in age groups under 50 years It is also striking that Doderlein s bacilli were still common in the postmenopausal age groups. This phenomenon may be attributed to the fact that the infection causes proliferation of the surface cells by which glycogen formation is sustained. An involvement of hormonal factors (a high oestrogen secretion) need not be presumed although this possibility cannot be ruled out with certainty

T Ne VII *Vaginal Bacterial Flora in Various Age Group of Series* (473 patients with Mycosis)

Age Group	–	16–	–25	26 30	–	36–40	–	46–50	51–55	56–60	61–65	66–70	70	Total Number of Cases
Number of cases		33	66	77	83	60	5	38	29	5	0	6	4	473
Doderlein bacilli		28	50	64	58	48	36	30	9	2	4	5		368
Coccoid flora		3		3	9	5	6				1		1	3
Mixed flora			6		6	7	9	6	9	3	5	1		75

Table VIII. *Clinical Diagnoses of 569 Patients (series 1, 2 and 3) with Various T of Mycosis*

	Yeast Species												
	Candida albicans	Candida krusei	Candida tropicalis	Candida parapsilosis	Candida reukaufii	Candida pulcherrima	Torulopsis glabrata	Torulopsis famata	Rhodotorula mucilaginosa	Rhodotorula pallida	Untyped	Total Diagnosed by Culture (Series 2 and 3)	Total Diagnosed by Vaginal Smear (Series)
Number of cases investigated	53	7	1	5	6	2	43	1	4	1	16	139	430
Colpitis, Cervicitis	29	3	1	4	4	2	21		1	1	4	70	115
Meno- metrorrhagia	3	1			1		2				4	11	29
Salpingitis							1					1	11
Neoplasms	7	1					6	1				15	67
Sterility	1						4				3	8	20
Pregnancy imminent or habitual abortion	3	1					3		1		2	10	82
Endocrine disorders	4	1					2					7	73
Uterine prolapse Urinary incontinence	3						1		1		1	6	33
Other	3			1	1		3		1		2	11	

The clinical diagnoses of the patients with mycosis appear in Table VIII. About half the number in series 2 and 3 and one fourth in series 1 had colpitis or cervicitis. No association with any particular disease was detected. It is striking that although *Torulopsis glabrata* usually occurred in a purer environment than *Candida* it was found in conjunction with inflammation of the vagina in 21 cases out of 70. Both for this reason and on the basis of our clinical experience it seems obvious that *Torulopsis* too may be pathogenic in the vagina (*cf.* Carter *et al.* 1959). When *Torulopsis glabrata* is pathogenic the rate of recurrence is high (Petru *et al.* 1956) and the infection may be very resistant to treatment.

The question of the criteria on which a clinical diagnosis of vaginal mycosis should be made is a matter for debate. Hesseltine (1959) did not regard a positive culture as sufficient evidence, since the carrier state is common. He maintained that a diagnosis of mycosis is justified only in cases showing obvious

Table IX, 6 *Cases of Vaginal Mycosis Classified According to the Morphological Finding in Vaginal Smears*

	Yeast Species											Total Diagnosed by Culture (Series ...)	Total Diagnosed by Vaginal Smear (Series ...)	Total Number of Cases
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Unknown			
Number of cases investigated	53	7		5	6		43		4		6	39	473	6 2
Few blastospores	5						5				4	19	93	
Blastospores in abundance	3	5					14					23	70	193
Few pseudomycelia	5											5	83	[illegible]
Pseudomycelia in abundance	2												69	7

clinical symptoms. In the present series of cultures signs of inflammation (which may however have been due to causes other than the presence of yeasts) were discernible in about half the cases. Hence it may be concluded that at least half the patients were asymptomatic. A similar result was obtained by Haberman et al. (1962) in a series of pregnant women. The rôle of yeasts in the ætiology of vaginitis and vulvovaginitis in particular is so important that a routine examination for this factor is always indicated. It may be recalled that Perl *et al.* (1955) detected Candida in 140 out of 350 cases of vulvovaginitis.

The morphology of fungi in vaginal smears

The morphology of fungi in vaginal smears is very stereotyped either bands or balls being seen (Table IX). As a rule *Candida albicans* forms pseudomycelia presenting as bands, but the absence of these does not rule out the presence of this species. Fig. 2 shows a typical instance of *Candida albicans* in a vaginal smear and Fig. 3 shows Candida krusei. It will be noted that the two species cannot be distinguished from each other. The blastospores of *Torulopsis glabrata* (Fig. 4) are somewhat smaller than those of Candida albicans but precisely identical forms

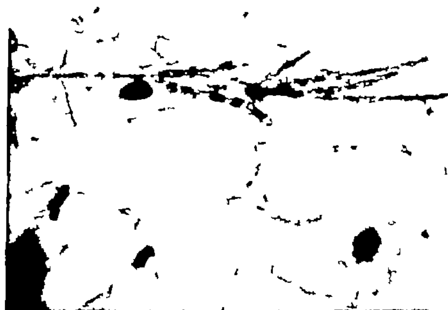


Fig 2 Candida albicans. Vaginal smear × 480.

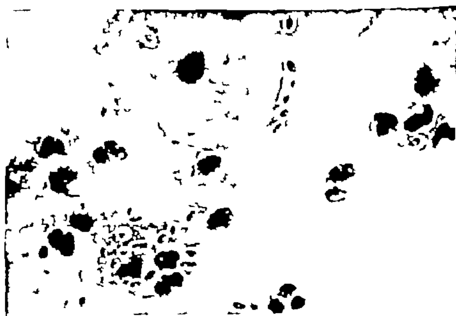


Fig 3 Candida krusei. Vaginal smear × 480



Fig. 4 Torulopsis glabrata Vaginal smear × 450.

are presented by *T. famata*. Hence differentiation of the various species is possible only with the aid of culture.

In conclusion it may be stated that by using Papanicolaou's technique as was done in the present study wet smear phase-contrast microscopy (Bourg, 1964) ordinary wet smear or the fluorescence method (van Niekerk, 1962) only one-seventh of the truly infected cases and of the carriers are detected. In order to detect mycosis it is not worth while therefore to use microscopic methods alone. A vaginal smear is taken in the first place to exclude malignancy not for the sake of diagnosing mycosis. Although a negative result does not exclude the possibility of mycosis, a positive finding may be useful when considering therapy

SUMMARY

1 The diagnosis, frequency and age distribution of mycosis and the relationship of this condition with various diseases are analysed in a series of 10 05 patients.

2. Yeasts were detected in cultures and vaginal smears in 118 out of 334 unselected gynæcological cases or 35.3 per cent. Among 10 344 patients examined only by vaginal smear 4.6 per cent exhibited mycosis. The conclusion is drawn that only about one seventh of cases of mycosis can be detected from preparations made by Papanicolaou's technique.

3. By culture *Candida albicans* was detected in 38.2 per cent, *Torulopsis glabrata* in 31.0 per cent of the cases of mycosis thus studied. Of less common yeasts the following were identified *Candida krusei* (5.0 per cent) *C tropicalis* (0.7 per cent) *C. reukaufii* (4.3 per cent) *C guilliermondii* (1.4 per cent) *Rhodotorula mucilaginosa* (2.9 per cent) *Rh. pallida* (0.7 per cent) and *Torulopsis famata* (0.7 per cent) In 11.5 per cent of the cases the nature of the infection remained unidentified.

4. Analysis of the age distribution of the patients with mycosis showed that this condition was most frequent in the group 31–35 years. In the age groups over 50 years the relative frequency of *Candida* increased as compared with the frequency of *Torulopsis*.

5. No clear correlation with endocrine disturbances was observed.

6 It appears that, apart from *Candida albicans Torulopsis glabrata* may be pathogenic in the vagina. In about half the cases in which yeasts were detected, symptoms of local infection were present.

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POST CÆSAREAN MENOURIA (YOUSSEF S SYNDROME)

BY

LAURI RAURAMO AND SEPPO RUPONEN

Case Report

The patient was a primigravida aged 22 whose last menstruation had occurred on April 25, 1963 The expected date of delivery was therefore Feb 2, 1964 and this was confirmed by the time of onset of foetal movements. Labour pains began on Jan. 26 1964, initially at intervals of 5 min., but disappeared gradually on the following day The patient then entered the maternity department of the Women s Clinic, University of Turku, where weak, irregular pains were noted. The laboratory findings were urine-albumin – sugar – urine-sediment negative B.P 120/80 T_{ax} 36 8° C. The foetal head presented, but was not engaged in the pelvic inlet, the external os admitted a finger there was no escape of amniotic fluid, the foetal heart rate was normal. As the patient was tired and the labour pains fairly weak, she was given 60 mg of Pethidine After she had slept part of the night the situation was unchanged. The patient developed coryza and her temperature rose to 38 8 C. The urine was negative leucocyte count 8,600, chest roentgenography was normal. As there was no progress in labour despite slightly stimulating medication, it was decided to perform a Cæsarean section on Feb 1 1964 A transverse lower segment operation was performed in the usual way the child weighed 3,300 g, the amniotic fluid was green and the consistency of porridge The mother s temperature on the first post-Cæsarean day was 36 6–36.8 C, but fairly profuse blood was seen in the urine and an indwelling catheter was inserted immediately Hematuria continued until Feb 12, 964, when the urine was yellow turbid, acid, erythrocytes totalled 4–6 and leucocytes 60–70 per field, bacterial staining was negative but E. coli grew on culture The urinary tract infection was brought under control and the patient was discharged on Feb 18, 964 At the follow-up examination on Feb 28 1964, hematuria was noted again and the patient was admitted to the Department of Gynæcology for treatment. The patient reported that she had been in good health, inconvenienced only by slight incontinence



Fig Cystography before operation =Bladder =Vagina 3 = Vesico-cervical fistula.

Cystoscopy was performed A fistula admitting finger was seen on the posterior wall of the urinary bladder above the left ureteral orifice When moderate amount of saline solution was introduced into the bladder part of it was seen to escape via the cervical canal and the external os The fistula wa lso visualized roentgenologically The patient was discharged under antibiotic prophylaxis to await uterine involution. Her condition was good after month at home and there was no longer any incontinence The first post partum menstruation ocurred on April 4, 964, when the menstrual discharge of blood was entirely via the urinary bladder without causing the patient any inconvenience After cessation of th menstrual period, operative repair of the fistula (Rauramo-Ruponen) was performed abdominally through lower median incision A fistula admitting finger easily wa found in the upper portion of the cervical canal at the level of the in ernal os, between the uterus and the urinary bladder After dissection the fistula wa losed in two layers, the bladder and the uterus being stitched separa tely The cervical canal was preserved and rubber tube wa led out from the external os The patient made good postoperative recovery and t later follow-up examinations clinical examination wa normal A mictur ting cystogram on May 29, 964, gave normal findings apart from slight reflux from he ureter The postoperative menses on May 4 and June 3 were normal



Fig 2 Cystography after operation normal condition.

Post-Cæsarean menouria of the type described above was first reported by Laffont and Ezes in 1947 The name menouria was however coined by *Abdel Fattah Youssef* in 1957 and the syndrome is known as Youssef's syndrome We have found only eight cases in the literature so the syndrome is fairly rare A common feature of all the cases is that they originated after one or more Cæsarean sections performed on the lower segment of the uterus. The Cæsarean section was performed via laparotomy in seven cases and in one case that reported by Ingelman Sundberg (1948) vaginal hysterotomy was carried out to remove a 23 cm long fœtus The urinary bladder had been damaged in all these operations in one way or another (thus an iatrogenic syndrome was involved) resulting in a vesico-uterine fistula This fistula had also developed inside the interior of the uterus which explains the symptoms characteristics of the fistula. After the post partum involution the internal os of the uterus blocks the cervical canal sufficiently to prevent the in-



Fig. 3 During the operation. =Uterus =Bladder The catheter passes into the bladder through the fistula

continence typical of ordinary vesical fistulas. Incontinence is a relative matter also in this syndrome and the patient does not necessarily notice it (unless exerting herself when the bladder is full) or at any rate it causes her no great inconvenience. When menstruation is resumed post partum the menstrual discharge naturally passes from the uterus along the easiest route and if there is a fistula of this type accumulates in the urinary bladder and escapes along this channel. The name menouria denotes, in fact, this cyclical massive haematuria. Vaginal discharge of blood during menstruation is rather scanty in the syndrome or hardly occurs at all. If no infection is present and the bladder does not become irritated during menstruation, the situation is actually quite fortunate: the patient is practically continent and hygiene during menstruation is much easier. In our case too the patient regarded the situation as fairly pleasant in this respect.

The menouria has fairly typical symptoms and is thus usually easy to diagnose. It must be remembered, however, that the



Fig. 4 During the operation a little later than in Fig. 3 1=Uterus, 2=Bladder The catheter is both in the bladder and in the cervical canal of the uterus

patient may develop as a sequel to surgery on the lower uterine segment vesical endometriosis and this naturally also leads to discharge of blood from the urinary bladder during menstruation. However vaginal menstrual discharge is predominant quantitatively and furthermore the patient with vesical endometriosis has pronounced symptoms of dysuria during the premenstrual period in consequence of irritation of the bladder Cystoscopy and hysterosalpingography are aids to diagnosis.

Operative repair of a fistula causing menouria is a fairly easy procedure and may be performed by either the vaginal or the abdominal route Youssef however held that abdominal surgery is basically superior and easier to perform since the fistula is localised fairly high in the uterus. In Hullemans case (1962) a patient of 42 was castrated by X rays as a therapeutic measure to terminate menstruation because the fistula was small and the patient s only symptom was cyclical discharge of blood via the urinary bladder menouria.

SUMMARY

A post-Caesarean vesico-uterine fistula is described with the typical symptoms relative incontinence and massive menstrual haematuria (menouria) Youssef's name is attached to the syndrome. Operative repair was performed by the abdominal route

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Fig. 4 During the operation a little later than in Fig. 3. 1=Uterus, 2=Bladder The catheter is both in the bladder and in the cervical canal of the uterus.

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TWO CASES OF SPONTANEOUS PERFORATION OF THE CÆCUM FOLLOWING CÆSAREAN SECTION[1]

BY

DAVID R. MILLAR[2] AND BJARNI OVLISEN

Perforation of the cæcum is rare and when it occurs it is usually a complication of local disease such as diverticulitis or carcinoma. It can also be an end result of overdistension of the cæcum, caused by obstruction of the distal colon in the presence of a competent ileo-cæcal valve (Saeltzer and Rhodes 1935 Ravid, 1951 Woodruff 1952 Albers *et al.* 1956 Lichtenstein *et al* 1961) In these circumstances the part played by ischæmia of the distended cæcal wall is illustrated by the fact that the perforation typically occurs on the anti-mesenteric border approximately 5 cm distal to the ileo-cæcal valve. When the large bowel is obstructed the intraluminal pressure is raised to the level of 12 to 52 cm of water (Wangensteen, 1955) Experiments on dogs show that if pressures of this order are maintained for 1 to 2 days in isolated loops of bowel areas of ischæmic necrosis can develop (Sperling, 1938) In the human, perforation is said to be imminent when the diameter of the cæcum reaches 9 cm as measured on a radiograph (Lowman and Davis, 1956)

[1] The first case was presented by one of the authors (DRM) at a meeting of the Section of Obstetrics and Gynæcology of the Royal Society of Medicine, London, on 28th Feb., 1964.

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Reports of caecal perforation complicating paralytic ileus are few and we have traced only four other cases in the literature. In one of these cases the ileus developed after Caesarean section (Robertson *et al.* 1958) and in two it was attributed to overwhelming infection (Morton *et al.* 1960). Caecostomy proved to be a life-saving procedure in these three cases. The fourth reported case was an alcoholic woman with multiple injuries who died without operation (Eckman *et al.* 1958).

The rarity of this complication prompts us to report two further cases in both of which ileus and caecal perforation followed Caesarean section.

Case

A 24-year-old primigravida with an immature and hysterical personality was admitted in early labour to the Birmingham Maternity Hospital. Her pregnancy had been uncomplicated but the foetal head remained unengaged. A standing lateral radiograph did not indicate disproportion; the true conjugate diameter measured 1 cm and the foetal skull was moulding well. The rectum was noted to be loaded with faeces so an enema was given, with good result.

The subsequent labour was characterized by disordered uterine action and was unusually painful. Analgesics and sedatives were prescribed liberally but, within 6 hours, a rising maternal pulse rate and the finding of acetonuria prompted the intravenous infusion of glucose. After 4 hours of ineffectual labour the cervix was still thick and only 3 cm dilated; after 24 hours it was half dilated but poorly applied to the foetal head. By that time the patient was exhausted and her bladder and colon were distended, so lower segment Caesarean section was carried out, using thiopentone, suxamethonium and nitrous oxide anaesthesia. The operation was uncomplicated and the blood loss was not excessive. A healthy male infant weighing 9 lb (4080 g) was delivered.

In the post-operative period, paralytic ileus developed but there was clinical improvement following an enema on the third day. Bowel sounds were heard. Two days later, however, the patient still complained bitterly of pain and tenderness over a distended caecum and showed signs of peritoneal irritation. Her temperature rose to 100·6° F (38 C). Radiographic examination of the abdomen at that time showed bowel distension typical of paralytic ileus and also a large amount of free gas under the diaphragm. Bowel perforation was considered but this diagnosis appeared unlikely because the woman was not hypotensive or dehydrated, and she never vomited despite taking adequate amounts of fluids with potassium supplements by mouth. Moreover, large amounts of flatus were passed by rectal tube. Urine output was good and the serum electrolyte levels re-

Millar David R and Ovlisen Bjarni Acta obst et gynec scandinav 294 1966

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TWO CASES OF SPONTANEOUS PERFORATION OF THE CÆCUM FOLLOWING CÆSAREAN SECTION[1]

BY

DAVID R. MILLAR[2] AND BJARNI OVLISEN

Perforation of the cæcum is rare and when it occurs it is usually a complication of local disease such as diverticulitis or carcinoma. It can also be an end result of overdistension of the cæcum, caused by obstruction of the distal colon in the presence of a competent ileo-cæcal valve (Saeltzer and Rhodes 1935 Ravid, 1951 Woodruff 1952 Albers *et al* 1956 Lichtenstein *et al* 1961) In these circumstances the part played by ischæmia of the distended cæcal wall is illustrated by the fact that the perforation typically occurs on the anti-mesenteric border approximately 5 cm distal to the ileo-cæcal valve. When the large bowel is obstructed the intraluminal pressure is raised to the level of 12 to 52 cm of water (Wangensteen 1955) Experiments on dogs show that if pressures of this order are maintained for 1 to 2 days in isolated loops of bowel, areas of ischæmic necrosis can develop (Sperling 1938) In the human, perforation is said to be imminent when the diameter of the cæcum reaches 9 cm as measured on a radiograph (Lowman and Davis 1956)

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intestinal perforation was made after further radiological examination with contrast medium in the colon.

At laparotomy a large quantity of offensive gas and faeces was found in the peritoneal cavity. On the anterior caecal wall an area of necrosis 8 cm. in diameter was seen and, in the centre of this, the bowel was perforated. Two similar smaller areas of necrosis in the ascending colon were noted. There were many fibrinous adhesions and the entire bowel was still distended. The pelvic organs appeared normal. Caecostomy was performed, and the peritoneal cavity drained. After operation the patient was in poor condition and despite the administration of antibiotics, blood transfusions, vasopressor drugs and steroids, she died the next day.

At autopsy the caecal wall around the areas of necrosis was indurated but histological examination failed to reveal any evidence of vascular thrombosis, malignant disease or ulceration which might have been responsible for the perforation.

Discussion

Under modern conditions of practice the immediate complications of Caesarean section are usually trivial. Severe degrees of paralytic ileus which formerly complicated classical section, often carried out in the presence of disturbed fluid and electrolyte balance, are now uncommon.

Why the patients whose cases are recorded above, suffered severe and persistent ileus is not clear.

In the first case the emotional make-up of the girl may have played a part. Before section, there was evidence of colonic distension associated with abnormal uterine action, but the electrolyte levels in the blood were normal. The paralysis of the bowel did not appear to be the result of puerperal sepsis as there was not any evidence of established peritonitis at laparotomy. The diagnosis of perforation was delayed because the signs of bacteraemic shock were absent, and the satisfactory outcome was probably explained by the fact that the unusually solid faecal content of the bowel prevented gross peritoneal soiling. The faeces may have caused an element of mechanical obstruction in the colon but the response to caecostomy alone suggests that this was of little importance. The conservative management of the ileus in this case was determined mainly by the patient's rejection of any active measures. Retrospective

mained within normal limits. Fluids were given intravenously and antibiotic therapy was commenced but tachycardia and pyrexia persisted and abdominal distension increased until by the seventh day the patient became alarmingly breathless. The volume of subdiaphragmatic gas, as shown radiographically had increased.

Laparotomy revealed a peritoneal sac grossly distended with gas which escaped in large amounts through the incision. The bowel itself was undilated and appeared healthy The peritoneal cavity was not contaminated with fæces but contained an odourless exudate reminiscent of that seen in association with gastro-duodenal perforation. Indeed, it was only after careful examination of the duodenum failed to reveal any abnormality that a tiny punched-out hole was found in the lateral wall of the cæcum. The colon was filled with fæces of a puttylike consistency but there was no sign of pre-existing cæcal ulceration. A cæcostomy was made and this drained for 6 days before bowel function returned to normal.

Subsequent complications included right-sided basal pneumonia associated with a subphrenic abscess, a pelvic abscess and epistaxis due to the administration of anticoagulants. Both abscesses required drainage The patient remained in hospital for some seven weeks but ultimately made a complete recovery

Case 2

A 35 year old secundigravida (one previous 12 week abortion) was admitted to the Rigshospitalet, Copenhagen, in labour at term Her pregnancy had been uncomplicated. After 1 hours of labour during which uterine contractions had been weak and infrequent, the cervix was only 3 to 4 cm dilated. The forewaters were therefore ruptured artificially Still labour did not progress satisfactorily so 7 hours later lower segment Caesarean section was carried out under suxamethonium, nitrous oxide and fluothane anæsthesia. A boy weighing 8 lb 6 oz (3900 g) was delivered without difficulty and in good condition. The blood loss at the time of operation was 800 ml. and this was replaced immediately There was no evidence of maternal ketosis.

On the second day after operation paralytic ileus developed and was treated by means of duodenal suction, neostigmine injections and enemata. On the fourth day the patient had a spontaneous bowel action and her general condition appeared satisfactory until, on the seventh day abdominal distension increased and she developed severe colicky pain i the right iliac fossa. Radiographic examination at that time showed generalised distension of the bowel and free subdiaphragmatic gas. The serum electrolyte levels were normal. A duodenal tube was again passed but without good effect Within a few hours of the onset of se ere pain, the patient's condition deteriorated rapidly Her temperature rose to 102.5 F (38.2 C) and the blood pressure became unrecordable A diagnosis of ileus complicated by

cecal valve is competent, the intra luminal pressure in the distended cecum will be raised significantly This sequence of events is a possible explanation both of increasing colonic distension despite the return of bowel sounds (in Case I) and of cecal perforation in the absence of true mechanical obstruction.

It seems that colonic ileus is liable to occur particularly when prolonged labour is terminated by Caesarean section and it is tempting to suggest that the sympathetic nervous system is, at least partly responsible for the disordered function of the uterus before delivery and of the large bowel during the puerperium.

In neither of the cases described here was there any evidence of pre-existing disease of the cecum. Nor was there any possibility of trauma to this area of the bowel at the time of Caesarean section. Self-retaining retractors were not used and, in the Danish case the intestines were protected by wet abdominal packs.

It may be added that the radiological sign of subdiaphragmatic gas was not accepted at the time as conclusive evidence of perforation in either of our patients. It was considered that it might represent air admitted at the time of Caesarean section. In fact subsequent radiological studies by one of us (DRM) have shown that air left in the abdomen at laparotomy is absorbed by the fifth day after operation.

SUMMARY

Two cases of spontaneous perforation of the cecum—one fatal—following emergency lower segment Caesarean section are presented.

Paralytic ileus resulting in cecal distension and ischaemic necrosis is considered t be the cause in both cases.

Acknowledgements

We are grateful to Mr Wilfrid Mills and Mr Arnold Gourevitch of Birmingham for their permission and encouragement to publish Case I

study however suggests that duodenal suction and intravenous feeding should have been started earlier

In the second patient the final clinical picture was one of bacteraemic shock caused by the presence of large amounts of faeces in the peritoneal cavity A more active line of treatment of the ileus in this case included the use of bowel stimulants and repeated enemata It is worth considering whether an enema could increase the intra-luminal pressure of an already ischaemic bowel sufficiently to induce perforation A presure of 100 cm (3 3 feet) of water is commonly used to introduce an enema—enough to impair significantly the blood flow to the bowel (Wangensteen 1955) That such pressures are sufficient to reach the caecum was shown by the diagnostic use of a contrast enema in this case It is probably not insignificant that in the two cases reported by Morton *et al* (1960) perforation of the caecum was diagnosed within 24 hours of barium enema.

It is well recognised that an element of mechanical obstruction often complicates and prolongs severe cases of ileus (Aird, 1957) Cannell and Tovee (1957) described 10 cases (eight following Caesarean section) in which the involuting uterus, sometimes firmly impacted in the pelvis obstructed the sigmoid colon. They were able to relieve the obstruction dramatically by rectal examination with the patient in the knee-chest position and they considered that the coincident volvulus of the colon in four of their cases was of secondary importance.

Three similar cases in which the uterus was the only recognised cause of colonic obstruction following Caesarean section were described by Antony and Wallace (1962) Laparotomy in each case showed that caecal perforation was imminent The pelvic organs were examined at laparotomy in both of our patients and in neither case was there any evidence of incarceration of the uterus in the pelvis.

Cases of segmental colonic ileus in the puerperium have been described (Laufe and Meyers 1955 Morton *et al* 1960) In these the paralysed segment acted as an obstruction to the flow of intestinal content. It is unlikely that all parts of the bowel recover from paralytic ileus simultaneously If peristalsis returns to the small intestine before the colon and if the ileo-

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CASTRATION IN EARLY PREGNANCY

BY

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As early as 1903 Fraenkel showed that castration or removal of the corpus luteum of pregnant rabbits was followed by abortion. In 1929 Allen and Corner confirmed the importance of the corpus luteum by showing that injection of corpus luteum extract into castrated rabbits preserved the pregnancy

However direct comparisons with man cannot be made. Several workers have described the successful outcome of pregnancy after removal of both ovaries or the corpus luteum (Essen-Möller 1904 Guldberg, 1936 Petersen, 1939 Kulseng-Hansen, 1951 Trolle 1955 Diczfalusy and Borell 1961 and others)

But the concept of the corpus luteum as a pregnancy-preserving factor cannot be dismissed entirely Apparently the earlier in pregnancy it is removed, the greater is the risk of abortion. Thus Ask-Upmark (1926) in a review of the literature found that 17 of 51 patients (33 per cent) aborted if the operation was performed within the first 2 months of pregnancy In a similar series of 20 patients Anderson and Stern (1963) found the incidence of abortion to be 23.5 per cent while Froewis (1963) concluded that castration within 8 weeks of the last menstruation almost inevitably resulted in abortion.

Therefore it would seem not to be of great interest to describe the uncomplicated course of a pregnancy in a patient who underwent bilateral oophorectomy 74 days after her last menstruation. However this paper is justified because during the re-

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Table I. *Excretion of Œstriol and HCG in Urine During Pregnancy Following Total Oophorectomy on 74th Day After Last Menstruation*

Day of Pregnancy	Urinary Output cc/24	Oestriol mg/24 h	HCG I. U./24 h
75	2080		400,000
76	030		150,000
8	900		60,000
83	070	6 5 μg	40,000
00	045	1.0	
07	952	1 7	
4	9 9		
121	875	3 0	
128	803	3 4	
30	898	9	3,500
35	000	3 9	5,000
142	37	4 5	0,000
149	1298	4 4	3,000
58	069	5 8	
63	930	7	
70	568	6	23,000
177	00	7 6	0,000
84	30	4 6	9,000
9	960	7 3	2,800
98	258	7 3	2,600
205	377	2	3,800
	878	5	35,000
9	070		,000
226	930	5	8,600
233	850	3	85,000
240	008	6 3	40,000
247	108	4 4	32,000
254	783	3 1	4 ,000
26	984	14 3	30,000
268	9 5	3 8	37,000
276	677	14	27 000
282	720	9 6	7,200
285		Delivery	
289	1700	5	700
292	626		300
317	363		300
33	358		50

mainder of the pregnancy frequent estimations of urinary oestriol and chorionic-gonadotrophin were carried out, together with vaginal cytology

Case history

A 22 year old married woman, who had not previously suffered from any serious illness, was admitted on 10.5.1963 for a suspected extra-uterine pregnancy She had menstruated regularly from the age of 13 years, menstruation lasting 4 days with 28 days between cycles her last menstruation began on 25.2.1963 At the time of admission there had therefore been 2 1/2 months amenorrhoea. Two years before she had had a normal delivery at term after an uncomplicated pregnancy She had had no abortions. A few hours before admission the patient experienced sudden severe pain in the left abdomen, she became generally unwell and nearly fainted.

Gynaecological examination on admission showed a small acyanotic cervix, displaced forwards with obliteration of the fornices. There was no bleeding from the cervical os. On manual exploration a large soft non-tender cystic swelling could be felt in the Pouch of Douglas extending to the right pelvic wall. On the left side one had the impression of a similar swelling stretching up to the level of the umbilicus The shape and position of the internal genitalia could not be determined with certainty Apart from the amenorrhoea there was nothing to indicate pregnancy

Because of the increasingly frequent and severe attacks of left-sided abdominal pain during the subsequent few hours, torsion of an ovarian cyst was suspected, and laparotomy decided upon. At operation the uterus was found to be enlarged equivalent to 8– o weeks gestation. The right ovary was replaced by a thin walled cyst containing 800 ml clear yellowish fluid The cyst was removed. On the left side was a similar cyst replacing the left ovary and extending up under the left costal margin. The pedicle was twisted through 360 and there was considerable enous congestion and oedema of the parametrium The cyst contained 3000 ml of clear yellowish fluid and was also removed

Histological examination showed that the cyst was pseudomucinous in type Ovarian tissue together with corpus luteum was also present

The postoperative period was uncomplicated and pregnancy continued normally No hormonal treatment whatsoever was instituted at this stage

On 7.12.1963 the patient delivered normally a living daughter in the right occipito anterior position (birth weight 3050 g, length 52 cm) The puerperium was normal

The hormonal investigation

No investigations were carried out before operation. Afterwards frequent estimations of the urinary oestriol and chorionic-gonado-

tion and the individual cell's glycogen content. Similary the acidophil index lay between 10–15 in the first 3 specimens, but fell to 2–3 in the 30th week, which is also normal

It was theoretically possible that some accessory ovarian tissue was left at operation and therefore the patient's excretion of pituitary gonadotrophin was followed after delivery These investigations were also carried out at the State Serum Institute in Copenhagen, using the method described by Johnsen (1958) The results are given in Table II. It would seem that the rising levels of excretion are proof that the patient was castrated. Because menstruation failed to re-establish itself and because of other deficiency symptoms cyclical substitution therapy was begun on 8.4.1964. The patient has since remained well and menstruated regulary The pituitary gonadotrophin excretion has also returned to normal.

Discussion

Castration or removal of the corpus luteum in pregnancy does not commonly occur That so radical an operation should have been performed is open to question but seen in the light of all the circumstances of the case it is thought to have been justified Previously published work on hormonal changes in pregnancy after castration are few

Amati (1928) Waldstein (1929) Szarka (1930) and Guldberg (1936) performed bilateral oophorectomy in the 4th month, 43rd, 80th, and 133rd day respectively after the last menstrual period. They all found normal post-operative oestrogen levels in blood and urine using biological assay methods. In contrast Allan and Dodds (1935) found (after bilateral oophorectomy on 112th day) lower than normal oestrogen levels during the remainder of the pregnancy Direct assays of oestrogen levels are thought only to have been performed by Diczfalusy and Borell (1961) and Mancuso (1962) Apart from a temporary post-operative fall in oestriol excretion in Mancuso's patient, these authors also found normal excretion of oestriol, 17β-oestradiol and oestrone later in the pregnancy

For practical reasons pregnandiol estimation was not possible

Table II. *Urinary Excretion of Pituitary Gonadotrophic Hormones After Normal Delivery of a Patient Castrated on 74th Day of Pregnancy*

Day After Delivery	Urinary Output cc/24 h.	Pituitary Gonadotrophic Hormones in /24 h.
16	1050	30
53	766	80
60	950	150
67	1080	240
74	1213	140
94	1693	248
101	1206	225
115	1017	250
122	940	350
Institution of cyclical treatment with oestrogens and gestagens		
136	983	198
150	1469	245
164	760	210
178	1198	140
192	1585	150
207	945	215
220	1060	100
276	1078	76
332	763	51

trophin excretion were carried out the dates of these can be seen in Table I

The analyses were carried out at the State Serum Institute in Copenhagen. The œstriol was measured by the method described by Aasted Frandsen (1963) Chorionic gonadotrophin was assayed biologically

As can be seen in Table I the œstriol and chorionic-gonadotrophin excretions were exactly the same as those found in a normal pregnancy

As stated above changes in the vaginal cytology were also studied. The method used was that described by Osmond Clarke and Murray (1958) The smears were taken in the 11th 13th, 19th and 35th weeks of pregnancy All the preparations were found to be normal with respect to the degree of cellular desquamation, navicular cell content, cellular agglutina-

tion in the immediate post-operative period, while others found the procedure to be without effect upon the urinary excretion of pregnandiol, oestriol 17-oestradiol, oestrone and chorionic-gonadotrophin. The author of one paper described a slight temporary fall in oestriol excretion immediately after operation.

As a result of the present work it is reasonable to assume that the hormonal environment in a clinically normal pregnancy occuring in a patient who has been castrated earlier in that pregnancy is identical with that found in a normal pregnant patient.

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in the present investigation but has been performed after oophorectomy or removal of the corpus luteum by Jones and Weil (1938), Seegar and Delfs (1940) and Trolle (1955). These workers all found a temporary fall to nil in the immediate postoperative period. Later pregnandiol was shown to be present by Jones and Weil but in decreased amounts.

Browne, Henry and Venning (1937), Tulsky and Koff (1957) and Mancuso (1962) found to the contrary that the operation was without influence on pregnandiol excretion, but Diczfalusy and Borell (1961) showed slightly increased excretion together with raised 17-hydroxy-keto-steroids which was thought to be due to the stress of operation.

Our patient had normal chorionic-gonadotrophin excretion until delivery and this agrees with the findings of Allan and Dodds.

We have been unable to trace earlier accounts of vaginal cytology following castration early in pregnancy. As previously stated the cytological findings were normal. Cytological changes in the vagina are presumably the result of total hormonal action on the vaginal mucous membrane. Our findings can therefore be taken as further evidence that castration performed on the 74th day after the last menstruation was without effect upon the hormonal balance of the pregnancy.

SUMMARY AND CONCLUSION

The case history is described of a patient who underwent bilateral oophorectomy during pregnancy (74 days after the last menstrual period). The pregnancy continued normally and resulted in the uncomplicated birth of a living term female infant. The castration was found to be without effect on the urinary excretion of oestriol and chorionic-gonadotrophin and the cytological changes in the vagina were also found to be normal throughout the remainder of the pregnancy.

These findings are compared with similar investigations following castration or removal of the corpus luteum in the first half of pregnancy (43–133 days after menstruation ceased). A few workers have shown a temporary fall in pregnandiol excre-

tion in the immediate post-operative period, while others found the procedure to be without effect upon the urinary excretion of pregnandiol oestriol 17-oestradiol, oestrone and chorionic-gonadotrophin. The author of one paper described a slight temporary fall in oestriol excretion immediately after operation.

As a result of the present work it is reasonable to assume that the hormonal environment in a clinically normal pregnancy occuring in a patient who has been castrated earlier in that pregnancy is identical with that found in a normal pregnant patient.

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MYOCARDIAL INFARCTION DURING DELIVERY

BY

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Myocardial infarction is uncommon in fertile women Mendelson (1952) in a series of 64 992 pregnant women seen in New York during the years 1932–50 found cardiac disease in 2 310 cases but coronary artery disease accounted for only 4 of these cases At the medical department of the Municipal Hospital of Kotka with an average yearly intake of 1,400 patients we have seen myocardial infarction in only three women under forty years of age during the last nine year- It is therefore evident that the combination of myocardial infarction and pregnancy is very rare

From the literature we have collected 35 cases of myocardial infarction during pregnancy or within one week *post partum* to which material we here add a case of our own (Table I) This patient is remarkable in that infarction occurred during delivery a situation we have not found described previously

Pregnancy after myocardial infarction seems rare too In the literature we have found only 11 such cases they are recorded without comments in Table II This rarity is probably partly to be explained by the fact that a woman having recovered from myocardial infarction usually escapes pregnancy in one way or another But another explanation could be that not all cases of pregnancy after myocardial infarction have been published

Former Head of the Obstetrical Department.
Head of the Medical Department.

Table I. *Myocardial Infarction During Pregnancy and Puerperium*

Author	Infarction			Termination		Survival of	
	Age	Pregn.	Month	Month	Manner	Mother	Child
Katz, 1922	33	Mult	V	X	Sudden death Autopsy	No	No
Reis 1935	44	4	X	X	Forceps	Yes	Yes
White 937	32	1	II	X	Forceps	Yes	Yes
Jensen, 938	38	4	V	X	Forceps	Yes	Yes
Hamilton, 94	?	6	II	III	Therap. abort.	Yes	No
Goldberger 950	37	5	IV	VIII	Spontaneous	Yes	Yes
Mendelson, 952	42		V	X	Section	Yes	Yes
Stewart, 952	42	?	V	X	Section	Yes	Yes
Stewart, 952	?		VIII	VIII	Death	No	No
Brock 953	34	4	II	X	Forceps	Yes	Yes
Antonius, 955	36		VII	VII	Forceps	Yes	Yes
Gordon, 1955		?	?	?	Death Autopsy	No	No
Gordon, 955		?	?		Sudden death Autopsy	No	N
Gordon, 955		?	?		Sudden death Autopsy	N	No
Levine, 958	37		Puerp	X	Spontaneous	Yes	Yes
Siegler 1958	38		II	X	Spontaneous	Yes	No
Forssell 957	38	3	II	X	Section	Yes	Yes
Forssell, 1957	44	Mult	VI	VII	Spontaneous	Yes	No
Massachusetts 957	4	4	X	X	Section post mortem, autopsy	No	Yes
Myers 957	39		II	X	Spontaneous	Yes	Yes
Oblo 958	35	6	Puerp	X	Section Autopsy	No	Yes
Urdan 959	3	6	Puerp	X	Spontaneous Autopsy	No	Yes
Vasick 959	2		Puerp	X	Spontaneous Autopsy	No	No
Brown, 960	3		Puerp	X	Spontaneous	Yes	Yes
Brown, 960	24		VI	VI	Sudden death Autopsy	No	No
Muir 960	28		V	VI	Sudden death Autopsy	No	No
Watson, 960	22		X	X	Section	Yes	Yes
Watson, 960	17		Puerp	X	Spontaneous	Yes	Yes
Jacobs 96	34	5	V	VII	Spontaneous	Yes	No
Naden 96	39	3	IV	X	Section	Yes	Yes

Phillips 1962	26	?	?	X	Spontaneous	Yes	Yes
Phillips 1962	?	?	?	?	?	Yes	Yes
Phillips 1962	?	?	?	?	?	Yes	Yes
Schapiro 1962	33	3	VIII	X	Forceps	Yes	Yes
Bechtel 1963	35	7	VIII	X	Section	Yes	Yes Yes
Listo 1966	28	2	X	X	Section	Yes	No

Case Report

Married bus-conductor born 1935. Heredity and past history uneventful. Serological tests for syphilis negative. Menarche at the age of 15, menses normal. Normal delivery of a healthy child in 1956. Last menstrual period in the beginning of June 1963. Weight gain from 54 to 73 kilos. Dyspnoea and a pressing retrosternal pain when moving from the end of Feb. 1964. She went into labour during the night of March 14, 1964, and came to the obstetrical ward of the Municipal Hospital of Kotka at 6 a.m. On admission labour appeared to be progressing satisfactorily and uneventfully. The foetal heart sounds were normal and labour pains occurred every 5–10 minutes. However, at 6,30 a.m. the patient suddenly developed severe pain in the chest and dyspnoea. After some minutes her condition improved, but at 7 o'clock the pain in the chest again became worse and she collapsed. At the same time a strong, painful and long uterine contraction occurred during which the foetal sounds ceased. An intravenous infusion of noradrenaline

Table II. *Pregnancy after Myocardial Infarction*

Author	Age	Infarction Years Before Pregnancy	Termination	Survival of Mother	Survival of Child
Horwitz 1943	35	One	Section	Yes	Yes
Laubach, 1951	40+	One	Therap. abort.	Yes	No
Laubach 1951	40+	One	Forceps	Yes	Yes
			(The mother died one year later)		
Levine 1951	22	?	?	Yes	Yes
Leff 1952	27	6/12	Forceps	Yes	Yes
Klein 1953	34	Two	Forceps	Yes	Yes
Lyons 1954	35	Two	Forceps	Yes	Yes
Burwell 1958	4	One	Spontaneous	Yes	Yes
	42	Two	Therap abort	Yes	No
Holoubek, 1959	39	5/12	Spontaneous	Yes	Yes
Holoubek, 1959	41	3/12	Section	Yes	Yes
Watson, 1960	23	One	Spontaneous	Yes	Yes
			(The same patient as in Table I)		

was started, and the blood pressure was maintained for most of the time at a level of 80–100 mm Hg; for short periods the blood pressure was unmeasurable. An electrocardiogram showed recent anterior myocardial infarction. The leucocyte count was 20,700, and there was no anaemia.

During the morning the patient was in a state of slight shock and complained of retrosternal pains and increasing dyspnoea. The pulse rate was raised, no murmurs wer heard; in the lungs soft rāles were heard and quite considerable quantities of bloodstained frothy sputum were expectorated. In spite of oxygen inhalation, injections of lanatoside C and morphine derivatives the dyspnoea became worse during th morning and several attacks of pulmonary oedema had to be relieved by tracheal aspiration. At the same time labour ceased. At 9 m. the cervix was not taken up but admitted 2 fingers At 1.45 p.m. it was still only fingers dilated but was half taken up

As the degree of cardiac failure became worse and the shock could not be overcome further delay was considered inadvisable. At 2.30–3 p.m. Caesarean section and tubal ligation were carried out under light ether anaesthesia. A male foetus weighing 3860 g. was stillborn. There were no malformations. The placenta was normal in appearance. The uterus showed no abnormality

The patient endured the operation well. Her pulmonary oedema disappeared by the time the uterine cavity had been emptied, and did not recur thereafter Yet slight shock persisted during the next 24 hours and there was transient oliguria with some proteinuria (0.4 g/litre). The serum non protein nitrogen value rose to 70 mg per oo ml and the serum creatinine to 3.5 mg per oo ml. The values returned to normal within days and the proteinuria disappeared The leucocyte count rose to 25,800 then fell to 7,800 The ESR rose to 100 and fell to 35 mm/h four weeks after the delivery The electrocardiogram showed changes typical of anterior myocardial infarction (Fig.) The patient was in hospital for four weeks and received dicoumarol for four months She went back to work in August, 964. She till is troubled by anginal pains on exercise.

Summary of the case

A 28 years old pregnant woman having had slight retrosternal pains for three weeks quite unexpectedly gets a myocardial infarction at the beginning of labour and becomes shocked. An abnormally strong uterine contraction follows and the foetus dies. After that labour ceases At the same time acute heart failure with pulmonary oedema appears. The failure is corrected immediately after Caesarean section the shock is overcome within two days. The patient recovers, but is still troubled by slight anginal pains.

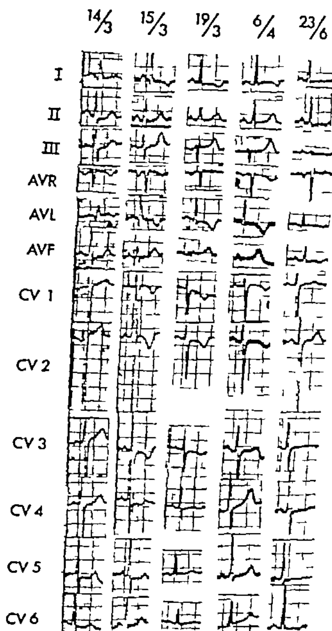


Figure 1 Electrocardiograms showing the development of an anterior myocardial infarction

Comments on the material in Table I

In this material the women's ages cover a wide range from 17 to 44 years. Six women had their myocardial infarction during the

first trimester ten during the second and eight during the last. In six cases the infarction took place in the puerperium In 11 women the infarction appeared during the first or second pregnancy in nine during the third to the fifth pregnancy Six women were in their sixth or later pregnancy

Eighteen women had circulatory troubles before the infarction. 12 of them had arterial hypertension, 11 anginal pains and five heart failure. Two women had syphilis and two diabetes.

Eleven of the women died. A woman having had myocardial infarction in the eighth month of pregnancy died later (Stewart, 1952) A woman with infarction in the fifth month died at the beginning of labour (Katz 1922) In nine cases death was sudden and unexpected the correct diagnosis was determined at the autopsy

In 13 cases the child was lost. In seven of these cases the mother died with the child unborn and in one case a therapeutic abortion was performed Three fortuses were born macerated one month (Forssell and Brunila 1957) six weeks (certainly alive three weeks earlier) (Jacobs and Moress 1961) and eight months (Siegler and Hoffman 1956) after the infarction. In our case the child died during labour and at the time of the infarction One other child died during labour the mother died four days later as a result of myocardial infarction (Vasicka and Liu, 1959)

Eight mothers died undelivered Records of delivery are to be found for 27 patients

With 13 women spontaneous delivery took place In eight of these cases both mother and child survived while three mothers survived delivery of a dead foetus. Two mothers suffered fatal myocardial infarction in the puerperium.

In six cases delivery was effected with forceps, with a good result for both mother and child.

Eight women underwent Caesarean section One of these had a fatal myocardial infarction in the puerperium (Maternal Health Committe Ohio 1958) One foetus died before section (our case) while in six cases both mother and child, twins in one case (Bechtel et al. 1963) survived.

Discussion

Coronary artery disease during pregnancy is not common but it constitutes a real problem which general practitioners, gynaecologists and internists may have to face This association is perhaps more common that would appear from the literature. In this connection we draw special attention to three patients in Table I (Maternal Health Committee Ohio 1958 Urdan and Madden 1959 Vasicka and Liu 1959) where the clinical picture showed postpartum shock perhaps due to pulmonary infarction but where autopsy revealed myocardial infarction One might ask in how many cases of postpartum shock where an electrocardiogram has not been taken and where autopsy has not been performed the shock has resulted from myocardial infarction We think that is the case not too rarely

One patient (Brown 1960) 24 years of age had complained of substernal pains on the day before her sudden death and had visited an emergency clinic where an electrocardiogram had not been taken This shows the importance of remembering the possibility of coronary disease during pregnancy

Despite the fact that many of the women in our collection withstood both infarction and pregnancy satisfactorily (however notes of the further development of the coronary artery disease are lacking in most cases) it seems evident that pregnancy is not a welcome situation for a woman with coronary artery disease. Contraceptive advice is certainly justified and even sterilization may be advisable

When myocardial infarction occurs during pregnancy the physician has to face many problems The first is how the infarction itself is to be treated The authors largely agree that general treatment and medication should be the same as it is in myocardial infarction without pregnancy It must be remembered that narcotics particularly during the immediately prepartum period can cause foetal respiratory depression. Narcotics therefore ought to be given with caution But on the other hand it is to be remembered that the very infarction pain through its shock promoting influence is injurious to the foetus Therefore we see no objection to giving large amounts of narcotics if the infarction pain is severe

Despite the fact that anticoagulant drugs have been used for myocardial infarction for almost 20 years the opinions on the justification and results are still at variance Anticoagulant therapy of myocardial infarction during pregnancy may create special problems. Both myocardial infarction and pregnancy predispose to thrombo-embolic disorders This combination may thus indicate the use of anticoagulants. On the other hand we know that certain anticoagulants can cross the placenta and cause lower prothrombin values in the foetus than in the mother Haemorrhagic complications can probably be avoided by cautious dosage without unduly lowering the prothrombin value

Eight of the mothers in our collection received anticoagulants They all survived the delivery One foetus was macerated, the others survived. On the other hand, in cases where anticoagulants were not given before delivery four foetuses died. This suggests that pregnancy is not in itself a contraindication to carefully controlled anticoagulant therapy

When the patient has survived the critical period after myocardial infarction the physician has to face two new problems. Is the pregnancy to continue? How is the delivery to be managed?

Of the 16 patients having myocardial infarction during the first and second trimesters 14 survived for more than one month. In one of them the pregnancy was interrupted because of arterial hypertension. One woman (Katz, 1922) died during delivery while 2 survived delivery Eight patients suffered myocardial infarction during the last trimester of the pregnancy Two of them died immediately the rest survived the delivery

These figures support the general opinion that myocardial infarction need not indicate interruption of the pregnancy

Our opinion is however that interruption of the pregnancy may be justified if the acute phase of the infarction is followed by persistent severe anginal pains or by a grave heart failure

Different opinions have been brought forth concerning the most suitable way of managing the delivery itself All opinions, including ours are based more on belief than knowledge

Generally it is considered that the delivery if it takes place spontaneously is to be facilitated and shortened by forceps or

Discussion

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fare Massachusetts, 1957) where five minutes after the mother had died suddenly from a recent myocardial infarction a living and undamaged child was delivered.

SUMMARY

A 28-year-old pregnant woman developed a myocardial infarction during delivery The foetus died and the labour ceased. The patient became shocked and acute heart failure developed. The shock and the failure disappeared following a Caesarean section performed eight hours after the myocardial infarction The patient survived. She is now two years after the infarction fit for work but suffers from slight anginal pains.

There are only 35 cases of myocardial infarction during pregnancy and puerperium reported previously Our patient is the first one to suffer myocardial infarction actually during labour

The treatment of myocardial infarction during pregnancy is discussed. Cautious anticoagulant therapy is permissible Therapeutic abortion is seldom indicated In most cases the delivery may proceed spontaneously but some aid may be needed in the final phase Yet in some cases a Caesarean section is the method of choice Preparations for resuscitation and postmortem Caesarean section should be made in advance

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vacuum extraction One patient (Reis and Frankenthal, 1935) was delivered successfully with forceps four days after the myocardial infarction

In most cases where Cæsarean section has been performed, this has been for indications other than the myocardial infarction itself Many authors (Brock *et al* 1953 Forssell and Brunila 1957 Mendelson 1952 Naden *et al* 1961 Schapiro *et al* 1962) consider that myocardial infarction *per se* is not an indication for Cæsarean section but that infarction on the other hand is no contraindication to section required for other reasons. However Watson *et al* (1960) consider Cæsarean section indicated after a myocardial infarction In their case section was performed two weeks after the infarction

The most difficult problem seems to be to choose the method of delivery when the myocardial infarction as in our case happens during delivery Initially we were inclined to let the delivery proceed spontaneously especially as the fœtus had died. When however labour ceased before full dilatation of the cervix and as there was a severe degree of heart failure we felt justified to use Cæsarean section The immediately favourable hæmodynamic consequences of this method reassured us of choice being correct in this case

The medical treatment of myocardial infarction during pregnancy should be directed towards maternal survival in this way we also give the child the greatest chance of surviving

We are of the opinion that every case of myocardial infarction during pregnancy requires individual obstetric assessment and treatment. Any generalisation for or against Cæsarean section is not justified Yet we think that section may be the best method of delivery in many cases

Coronary artery disease carries a risk of sudden death through venticular asystole or fibrillation Every time a woman suffering from coronary artery disease or myocardial infarction is to be delivered everything should be ready for resuscitation and for immediate Cæsarean section If the resuscitation does not lead to an immediate response Cæsarean section can save the child This has happened in one case (Committee on Maternal Wel

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ÆTIOLOGICAL FACTORS IN PREMATURITY

BY

INGE JANSSON

Introduction

The obstetrician of today enjoys a greatly reduced maternal mortality and also some reduction in perinatal infant mortality The latter is, however far less pronounced than the former Thus Bartsch and Bergman (1964) from the Women's Clinic II in Gothenburg reported a decrease of perinatal mortality from 2.6 per cent during the period 1951–1953 to 1.9 per cent during the period 1960–1962 of the following decade We can now expect a perinatal mortality around 2 per cent, a figure which is difficult to improve upon. The reason for this is that the majority or about 60 per cent, of the infants dying perinatally are born prematurely During the past decade the prematurity rate has remained constant at about 5 per cent of the number of infants born. Approximately one fourth of the premature infants are either born dead or die within one week after delivery This figure has also remained fairly constant, although a slight decrease has been noted (Table 1)

The obstetrical results can of course not be measured only by mortality figures The aim of the obstetrician is to deliver a healthy infant capable of developing normally We know that premature children especially in the lower weight groups get a bad start in life and that in many cases they are never able to catch up with the mature children. This is one of the principle reasons why one of the most important tasks in obstetrics is to study the causes of prematurity and to try to find ways of preventing the growing foetus from being expelled from the uterus too early

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The obstetrical results can of course not be measured only by mortality figures. The aim of the obstetrician is to deliver a healthy infant capable of developing normally. We know that premature children especially in the lower weight groups get a bad start in life and that in many cases they are never able to catch up with the mature children. This is one of the principle reasons why one of the most important tasks in obstetrics is to study the causes of prematurity and to try to find ways of preventing the growing fœtus from being expelled from the uterus too early.

Table 1 *Rate of Prematurity and Perinatal Mortality of Premature Infants at Women's Clinic II Gothenburg*

	1954—56	1957—59	1960—62	1963—65
Total No. of infants born	6793	6772	6277	7357
No. of premature infants	341	320	317	394
Prematurity rate %	4.9	4.7	5.0	5.4
No. of perinatal deaths	89	79	71	76
Perinatal mortality %	26.1	24.6	22.4	19.3

Only about 40 per cent of premature births can be explained by any of the known causes of prematurity. In this context the expression "known cause" refers only to such obvious factors as severe maternal toxæmia, obstetric hæmorrhage caused by placental complications such as abruptio placentæ and placenta prævia, multiple pregnancy and malformation of the child, which are all factors known to produce a high incidence of premature births. During recent years other and less obvious factors have been shown statistically to be connected with prematurity. Such factors are the socio-economic back ground of the parents and maternal age and parity. Genetic factors also have some importance. There is a correlation between maternal height and weight and the birth of a premature infant, *i.e.* small women give birth to premature infants more often than their taller sisters. Furthermore Räihä and Unnérus (1956, 1959) in Finland have demonstrated that women with a small heart volume assessed rœntgenologically show a higher prematurity rate than those with a normal heart volume. It has also been shown recently that urinary infection in pregnancy (Kass 1960) and cigarette smoking in pregnancy (Simpson, 1957) increase the premature rate.

Material and Methods

A series of mothers of premature infants from the Women's Clinic II in Gothenburg has been analyzed to investigate to what extent it is possible to improve our results rather than in the hope of contributing anything new to the ætiology of prematurity.

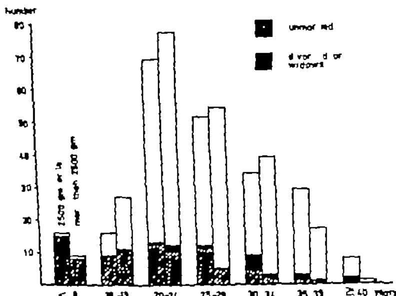


Fig. Distribution according to age and marital status of 223 mothers of infants with birth weight 2500 g or less and of 223 mothers of infants with birth weight over 2500 g

All mothers of infants with a birth weight of 2500 g or less, born during the two year period from March 1 1962 to February 28 1964, were interviewed within a few days after delivery with regard to factors of possible significance for premature birth. With few exceptions the interviews were performed by the author and the details recorded on a special form. The series comprises 223 mothers of 240 premature children. Unfortunately no control series was collected at the same time and by the same method. Instead a control series of the same size was obtained subsequently. It consists of all mothers of infants with a birth weight of more than 2500 g and with record numbers next to the mothers of the premature infants. Consequently this material gives information only of such factors that are registered in the case records. When admitted to the delivery ward these mothers were interviewed by the student nurses of the midwifery school according to a special scheme printed in the case record.

Results

Age and marital status

Figure 1 illustrates the age distribution of mothers of premature babies and of mothers of mature babies Twice as many of the mothers of premature infants were under 18 years as in the control series (7 2 per cent and 3 6 per cent, respectively) and twice as many were 35 years of age or over (16 6 per cent and 8.1 per cent respectively) In the age groups between 19 and 34 years there was no demonstrable difference. On the contrary the group 18–19 years was smaller in mothers of premature infants than in the control group

The marital status of mothers also is clear from figure 1 Fifty two mothers of premature infants were unmarried and 11 were divorced or widows. The corresponding figures for mothers of mature children were 36 and 3. Altogether 28 2 per cent of the mothers of premature infants were unmarried, divorced or widows while the corresponding figure for control mothers was 17 5 per cent

Socioeconomic class

The material was divided in three socioeconomic classes. The classification was based on the husband s and the mother s occupation and the education of the parents The results are shown in fig 2 Class I constituted a very small part of both series. Class II was preponderant in mothers of mature children while most mothers of the premature infants belonged to the least favoured class III An equal number of mothers (14) were of foreign nationality in both groups.

Obstetric history

One hundred and eleven of the mothers of premature infants and 8_ of mothers of infants with birth weight over 2500 g were primigravidæ Out of the 112 multigravidæ in the premature series 30 (26 8 per cent) had had premature deliveries before 43 (38.4 per cent) had sustained spontaneous or legal abortion and 15 (13 4 per cent) had born infants which died perinatally

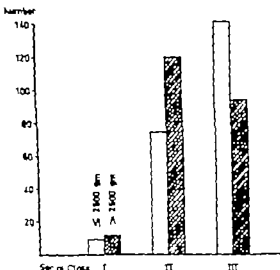


Fig. Distribution according to socio-economic class of 223 mothers of premature infants and of 223 mothers of mature infants.

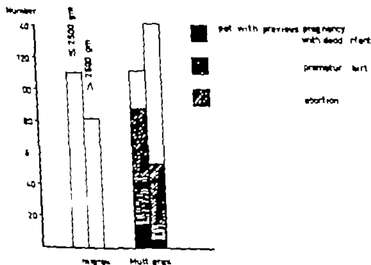


Fig. 3 Distribution of mothers of premature infants and mature infants by gravidity. In multigravidae the outcome of previous pregnancies is indicated.

Results

Age and marital status

Figure 1 illustrates the age distribution of mothers of premature babies and of mothers of mature babies. Twice as many of the mothers of premature infants were under 18 years as in the control series (7.2 per cent and 3.6 per cent respectively) and twice as many were 35 years of age or over (16.6 per cent and 8.1 per cent respectively). In the age groups between 19 and 34 years there was no demonstrable difference. On the contrary the group 18–19 years was smaller in mothers of premature infants than in the control group.

The marital status of mothers also is clear from figure 1. Fifty two mothers of premature infants were unmarried and 11 were divorced or widows. The corresponding figures for mothers of mature children were 36 and 3. Altogether 28.2 per cent of the mothers of premature infants were unmarried, divorced or widows, while the corresponding figure for control mothers was 17.5 per cent.

Socioeconomic class

The material was divided in three socioeconomic classes. The classification was based on the husband's and the mother's occupation and the education of the parents. The results are shown in fig. 2. Class I constituted a very small part of both series. Class II was preponderant in mothers of mature children, while most mothers of the premature infants belonged to the least favoured class III. An equal number of mothers (14) were of foreign nationality in both groups.

Obstetric history

One hundred and eleven of the mothers of premature infants and 82 of mothers of infants with birth weight over 2500 g were primigravidæ. Out of the 112 multigravidæ in the premature series 30 (26.8 per cent) had had premature deliveries before, 43 (38.4 per cent) had sustained spontaneous or legal abortion and 15 (13.4 per cent) had born infants which died perinatally.

Table II. Occurrence of *Toxæmia in Mothers of Infants with Birth Weight 2500 g or less and over 2500 g*

Type of Toxæmia	No. of Mothers of Infants with Birth Weight ≤ 2500 g	No. of Mothers of Infants with Birth Weight > 2500 g
Albuminuria (Esbach < 1 ‰) and/or hypertension	32	3
Albuminuria (Esbach ≥ 1 ‰) and hypertension	8	
Severe pre-eclampsia	1	
Eclampsia		1
Total	42 (18.8 %)	16 (7.2 %)

mild or severe nausea and vomiting during pregnancy. Anæmia was registered in 26 and 36 respectively. Thus, the difference, if any, was to the disadvantage of the mothers of term babies.

Hæmorrhage in any stage of pregnancy was a common symptom in the mothers of premature infants. Forty-five of these (20.2 per cent) had noticed bleeding of some degree and 9 had been treated in the hospital because of threatening abortion. The corresponding figures for the controls were 14 (6.3 per cent) and 1 respectively. Among mothers of term babies the hæmorrhage was usually very slight and interpreted as coming from a cervical erosion.

Fifteen mothers of premature infants and 12 mothers of term infants had been treated for clinically overt *urinary infection* during the current pregnancy. Thus there was no difference in this respect.

Toxæmia. Only distinct cases of toxæmia were counted. Cases of mere œdema and abnormal weight gain were discarded as the registration of these cases is uncertain. The results are shown in Table II.

There was a great preponderance among mothers of premature infants of all forms of toxæmia except eclampsia, of which there was one case in each group.

In 16 premature births there were *placental and umbilical cord complications.* These cases are analysed below. There was no such case in the control group.

The corresponding figures for the 141 multigravidæ of control mothers were 10 (7.1 per cent), 38 (27.0 per cent) and 5 (3.5 per cent). The results are shown graphically in figure 3. There is a clear difference in the proportion of primigravidæ to multigravidæ and for previous premature births and perinatal deaths, while the difference in the number of mothers with previous abortions is less striking.

General and gynæcological history

Sixty-one mothers of premature babies (28.6 per cent) and 34 mothers of full term babies (15.2 per cent) had earlier suffered from some general disease. There were a few cases of pulmonary tuberculosis, rheumatic disease and valvular heart disease in both groups. The difference was mainly due to disorders of the urinary system, mostly pyelonephritis and cystitis, which had affected 41 (18.4 per cent) of mothers in the premature group and 25 (11.2 per cent) in the control group. There is a possibility of methodical error, as the mothers of the premature infants were questioned especially with regard to urinary infection. On the other hand, previous urinary infection was included in the case history of the obstetric records, and thus all mothers were interviewed with regard to past urinary infection.

Fifty-five mothers of premature infants and 40 control mothers had sought medical advice for gynæcological disorders. In 23 and 24 cases, respectively, the diagnosis was salpingitis and/or gonorrhoea and in 15 and 13 cases, respectively, varying gynæcological disorders such as menstrual abnormalities, ovarian cysts and myomas. There was a definite difference only concerning previous sterility. Thirteen mothers of premature infants had a history of infertility (5.8 per cent) as compared with 3 control mothers (1.3 per cent).

Current pregnancy

There was about an equal number of cases of *emesis-hyperemesis* and *anæmia* (hæmoglobin below 10 g. at any occasion during pregnancy) in both groups. Fifty-eight mothers of premature babies and 70 mothers of term babies had suffered from

In 100 cases (44.8 per cent) the cause of the premature birth was fairly apparent (Table III)

In Table III the cases are classified according to the factor which was judged to be the main cause of the premature birth. Toxæmia was the largest group and the second most common causative factor was multiple birth, with 27 cases (12.1 per cent of the total number of premature births) Among the term pregnancies there was only one set of twins, and the overall incidence of multiple births in our clinic is only slightly above 1 per cent. Placental and umbilical cord complications and foetal malformation also were common causes with 16 and 15 cases respectively It may be questioned whether it is justified to assume mild toxæmia to be the only cause of a premature birth. It is obvious that more than one causative factor is responsible in many cases.

The cases of Cæsarean section before term demand some comments

The first case was 35 years old primigravida with mitral valvular disease She was admitted to the hospital because of weak pains. The duration of gestation was uncertain but she was considered to be around term. Cæsarean section was performed because of primary uterine inertia The birth weight of the infant was 300 g. The other case was a 33 years old primigravida with previous infertility of several years duration She was admitted to the clinic in the 32nd week of gestation with mechanical intestinal obstruction, which required immediate surgical intervention In order to separate the extensive adhesions it was necessary to empty the uterus The infant weighed 930 g and died after 4 hours

In 123 cases (55.2 per cent) there was no generally accepted cause for the premature birth. It is reasonable to assume that the above mentioned general causative factors also played a role in this group of premature births of unknown ætiology Twelve out of 16 mothers under 18 years belonged to this group but mothers aged 35 years or more were equally distributed in the "known and unknown cause groups. There was a preponderance of social class III in the unknown group 86 out of 123 (70 per cent) compared with 54 out of 100 (54 per cent) with known causes. Seventeen women had sustained serious psychical strain, while only 4 of those with serious obstetric complications

Table III *Number of Cases with Apparent Cause for the Premature Birth*

Toxæmia	
albuminuria < 1 ‰ and/or hypertension	14
albuminuria ≥ 1 ‰ and hypertension	6
severe pre-eclampsia	11
Subtotal	31
Twin pregnancies	27
(9 out of which with toxæmia)	
Placental and umbilical cord complications	
toxæmia with abruptio placentæ	4
abruptio placentæ without toxæmia	1
placenta prævia	7
true knot of the umbilical cord with intrauterine fœtal death	1
umbilical cord strangulation with intrauterine fœtal death	1
massive placental degeneration	2
Subtotal	16
Severe fœtal malformation	15
Cervical incompetence	5
Rh or ABO iso-immunization	2
Maternal syphilis	2
Caesarean section before term	2
Total	100

Prematurity of known versus unknown aetiology

In addition to the data accounted for above the mothers of premature infants were interviewed with regard to smoking, occupation and psychical strain during the pregnancy. There are no control data available for these factors. One hundred and eleven were cigarette smokers (49.8 per cent), 112 (50.2 per cent) went out to work during the greater part of the pregnancy and 23 (10.3 per cent) had been the subject of some obvious psychical strain (death of close relative, severe protracted psychical strain). As will be mentioned later these figures have a certain interest in spite of the absence of a control series.

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Table V *Distribution of Smoking Mothers of Premature Infants in Different Socio-economic Classes*

Class I	3 smokers out of	9	(33 %)
Class II	30	74	(41 %)
Class III	78	140	(56 %)

Table V shows the distribution of smoking mothers by social class.

The numbers in class I are too small to permit any conclusions It is worth mentioning, however that in 8 of these 9 patients there was a definite explanation for the premature birth. The difference in the proportion of smokers in social classes II and III is obvious.

Among the cases of prematurity of unknown ætiology there were 11 cases where a constitutional or genetic factor may have been operative These mothers were either extremely small of stature themselves and the birth weight of their infants at term was slightly less than 2500 g or they had previously born several infants just under 2500 g at term. These infants cannot be regarded as premature in the true sense

Urinary tract infection was diagnosed in 8 of the cases with prematurity of "known cause (8.0 per cent) and in 18 of the group with unknown ætiology (14.6 per cent) The figures are too small to permit any conclusions.

The premature infant

The 223 premature labours resulted in 240 premature infants. There were 27 twin pregnancies, but in 10 of these only one twin weighed 2500 g or less. The birth weight, length of gestation and perinatal mortality of these 240 infants are shown in Tables VI and VII.

In Table VIII the 51 perinatal deaths are classified according to obstetric factors of significance for prematurity and foetal death

Apart from the 9 malformed infants who died, there were 8 other premature infants with malformations. All premature in-

Table IV *Relationship of Number of Smoking and Non-smoking Mothers of Premature Infants to Duration of Gestation*

Duration of Gestation	Smokers	Non-smokers
Less than 29 weeks	6	6
29–32	12	21
33–36	30	42
37–40	54	28
More than 40	8	14
Uncertain	1	1
Total	111	112
No. of perinatal deaths without malformation	9	33

admitted psychical strain of any significance. Occupational work was equally common in both groups

Seventy-nine out of the 123 mothers with unexplained premature birth were cigarette smokers (64.2 per cent) compared with 32 (32.0 per cent) mothers with obvious obstetric cause for premature birth Several investigators have shown that smoking in pregnancy does not shorten the gestational period but that the average birth weight of infants of smoking mothers is less than that of infants of non-smoking mothers. In Table IV the number of smokers and non-smokers is given in relation to the length of gestation.

Sixty-two of the smokers (55.8 per cent) had a gestational period of 37 weeks or more, while 42 of the non-smokers (37 5 per cent) reached the same period. The number of perinatal deaths is included to illustrate that not only was the average gestational period shorter in non-smoking mothers of premature infants but also that the perinatal mortality rate was higher in this group

The relationship can be elucidated from another point of view Out of 85 mothers of infants with birth weight 2001–2500 g and a gestation of 37 weeks or more, 50 (59 per cent) were smokers Out of 53 mothers of infants in the same weight group but a gestation less than 37 weeks 17 (32 per cent) were smokers.

Table IX. Malformations in Premature and Mature Infants

Birth Weight ≤ 1900		Birth Weight > 1900 g	
Acrania	4	Œsophageal atresia	
Myelomeningocoele		Congenital heart disease	1
Microcephaly	3	Syndactyly or polydactyly	3
Multiple malformations	3	Hypospadia	
Mongolism	2		
Congenital heart disease	2		
Congenital megacolon	1		
Total	17 (7.6 %)		6 (7 %)

fants with malformations are detailed in Table IX and are compared with the mature infants with malformations.

Among the mature infants there were only two with serious mal formation, while all 17 premature infants had gross malformations

Table VIII demands a few comments.

The case of habitual intrauterine foetal death was 32 years old gravida III with one previous late abortion and one premature birth with dead infant. She was delivered in the 37th week of a macerated female infant with a birth weight of 400 g The placenta was strikingly small. It weighed 220 g and measured X cm The case must be designated as placental insufficiency with malnutrition of the foetus of unknown cause

The syphilitic case was 7 years old unmarried gravida I Positive Wassermann and Treponema pallidum immobilization tests were discovered at delivery She had not received any treatment during pregnancy She was delivered in the 3 st week and the infant weighed 1440 g It died 24 hours post partum Post mortem examination revealed pulmonary atelectasis but no certain evidence of congenital syphilis Thus other factors may have been responsible for prematurity and foetal death

The largest group of perinatal deaths was uncomplicated immaturity These infants had immaturity as a sole diagnosis Six of them were twins In the rest of the cases no acceptable cause for the prematurity could be established.

Discussion

The aetiology of prematurity is a problem of an extremely complex natur There is an interaction of many different factors

Table VI *Relation of Perinatal Mortality to Birth Weight of Premature Infants*

Birth Weight	No. of Infants Born	Perinatal Deaths	Mortality per Cent
1000 g or less	13	11	85
1001–1500 g	26	18	69
1501–2000 g	59	13	22
2001–2500 g	142	9	6.3
Total	240	51	21.2

Table VII *Relation of Perinatal Mortality of Premature Infants to Duration of Gestation*

Duration of Gestation	No. of Infants Born	Perinatal Deaths	Mortality per Cent
Less than 29 weeks	13	9	69
29–32	38	19	50
33–36	80	15	19
37–40	86	6	7
More than 40	21	1	4.8
Uncertain	2	1	
Total	240	51	21

Table VIII *Causes of Prematurity and Foetal Death in 51 Premature Infants Who Died in the Perinatal Period*

Diagnosis	Dead Ante Partum	Dead Intra Partum	Dead within One Week Post Partum
Malformation	6		3
Toxemia (cases with abruptio placentae included)	6		2
Placental and umbilical cord complications	6		2
Cervical incompetence			2
Habitual foetal death	1		
Rh immunization	1		
Syphilis			1
Operation for ileus in mother			
Uncomplicated immaturity	4	1	14
Total	24	2	25

and several others have shown that women under 20 or over 30 years of age give birth to more premature infants than do women between these ages. In our series the influence of age was only obvious under 18 years and at 35 years and over. Among mothers of premature infants there were twice as many women under 18 and also twice as many women 35 years of age or more as compared with the control group. It is noteworthy that the age group 18–19 years was, on the contrary, smaller among mothers of premature infants. Thus it seems that only the youngest teen-age mothers have an increased risk of premature birth. This may be connected with the fact that the teen-agers are a special group, where a bad nutritional state as well as a possible biological immaturity may be of importance. In the older mothers other factors such as toxaemia superimposed on preexisting hypertension are at work.

Premature birth is more common in a first delivery than subsequently with the possible exception of an increase at parity five or more. Among our mothers of premature infants there was a preponderance of primigravidæ. The size of the series did not permit division according to birth number. The varying rate of prematurity in relation to parity is of course connected in part with the age of the mother. The age, however, seems to be more important for the prematurity rate than the birth number (Donnelly et al. 1964).

The women who gave birth to premature infants were more often unmarried, divorced or widows than those who gave birth to mature children (28.2 per cent and 17.5 per cent, respectively). This can in part be explained by the fact that young unmarried mothers and elderly divorced mothers were more common in the premature series. There seems, however, to be a preponderance of unmarried also in the intermediate ages. It is reasonable to assume that a difficult social situation with severe psychical pressure can contribute to the induction of premature labour. This question is elucidated by the fact that 12 per cent of the women belonging to the group of prematurity of unknown etiology had sustained serious psychical strain during pregnancy as compared with only 4 per cent of those with known cause of the prematurity.

In this paper no attempt has been made to support the differences found with statistical calculations as one factor may have an influence on another as for instance age on marital status. The established relationships are not necessarily causative. Only in a limited number of cases can a direct cause for the premature birth be established. In this series obstetric complications such as toxæmia placenta prævia abruptio placentæ multiple pregnancy and fœtal malformations comprised 45 per cent of the total number of cases This figure agrees well with previously published results of others In these cases it can be maintained that the complication in question was the immediate cause of the premature birth In many cases however more than one factor has been active Combinations such as those of toxæmia and multiple birth and of toxæmia and placental complications are common

In more than half the mothers of the premature infants, however complications with immediate relation to the prematurity was missing This does not mean that we are completely ignorant of the cause in these cases. General social and obstetric factors are of importance in this connection. Donnelly Flowers Creadick Wells Greenberg and Surles (1964) have shown in a large American series that the prematurity rate increased from 5 per cent in social class I to 14 7 per cent in social class IV The classification was based on the occupation of the husband and the education and race of the woman. This corresponds with the findings in our series where 63 per cent of mothers of premature infants belonged to social class III while only 42 per cent of mothers of mature infants belonged to this class. In what way the socioeconomic background of the mother influences the prematurity rate is not clear Terris (1965) has suggested that a poor nutritional state among the lower classes should affect the prematurity rate This proved very difficult to verify The only consistant finding was a marked increase in the rate of prematurity when the daily protein intake was less than 50 g It is hard to believe that this could occur in Sweden today

Maternal age parity and marital status are factors which have an influence on the rate of prematurity Donnelly *et al* (1964)

ledge of the ætiology of toxæmia and better therapeutic methods.

The second largest ætiological group in our series was twin pregnancy which accounted for 17 per cent of the premature infants. In twin pregnancy there is sometimes a connection between the two circulation systems which may cause one foetus to grow to the detriment of the other Such an infant may be extremely small and yet born at term. Most premature twins, however are premature by weight as well as by gestational age. In these cases the cause of the premature labour may be the excessive uterine distension. Kærn (1962) in Denmark, among others has shown that it is possible to reduce the rate of prematurity and perinatal mortality by admitting women with multiple pregnancy to hospital The problem is of course to establish the diagnosis early enough in pregnancy Too often the diagnosis of twins is not made until delivery

Placenta prævia and abruptio placentæ without coincident toxæmia was the cause of the prematurity in 5 per cent of the cases. Placental complications of this type are sometimes revealed by hæmorrhage early in pregnancy Hæmorrhage from these causes or as a sign of imminent abortion or premature labour was three times more common among mothers of premature infants than among those of mature infants. This gives certain possibilities for prophylaxis. Even in placenta prævia it is possible to gain time by strict bed rest.

The significance of urinary infection in pregnancy as a cause of prematurity has been the subject of discussion. Kass (1960) reported asymptomatic bacteriuria in 6 per cent of pregnant women and found that 40 per cent of these women developed a pyelonephritis during pregnancy or the puerperium. He also observed that untreated bacteriuria was connected with a high frequency of prematurity and perinatal death. Several investigators have found about the same rate of bacteriuria in pregnancy as Kass but they have not been able to confirm his results regarding the rate of prematurity (among others Chalmers, 1963 Forkman, 1964) Quite recently however Kincaid-Smith and Bullen (1965) Stuart, Cummins and Chin (1965) and Rannevik (1965) ha e published results which agree with the original observations of Kass.

It is evident that the mother of a premature infant is often *a primipara and that she is often in an unfavourable social* situation. When it comes to the multiparæ another interesting fact appears. They constitute an obstetrically afflicted group, who have given birth to premature and perinatally dead infants three times more often than the mothers of full term babies. Thus the factor which was once responsible for the premature birth has a tendency to repeat itself in the following pregnancies. *In certain cases this factor may* be of a genetic or constitutional nature. This is valid for women of small stature whose infants have a birth weight under 2500 g despite a normal gestation period. These infants are not really premature and behave subsequently as term babies. In other cases it may be a matter of an obstetric complication which repeats itself or an obstetrical short-coming which it is not possible to analyse. Habitual intra uterine *foetal death is a rare but well known obstetrical* entity

In this connection so called cervical incompetence is of interest as a cause of abortion and premature labour The diagnosis is always uncertain in these cases Five of our premature births were regarded as due to this cause It is probable that intense prophylactic therapy with cervical cerclage and prolonged bed rest forced these pregnancies beyond the abortion limit These *pregnancies resulted in six infants of which it was possible to* save three

The largest causative group was in ours as well as in other series of premature births toxæmia a complication which appeared in almost 19 per cent of the mothers of premature infants and often in its most serious forms. Toxæmia is associated with placental insufficiency and impaired nutrition of the *foetus and the gestational period is shortened* either spontaneously or because of the intervention of the obstetrician. Consequently this complication makes the greatest contribution of infants in the lowest weight groups with the worst prognosis. It is probable that early *admission to hospital in mild* cases of toxæmia can prevent progression of the disease In acute severe toxæmia with its rapid course we are still rather powerless. Essential improvements in the rate of prematurity and perinatal mortality in this group can only be reached with deeper know-

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Thus Kincaid Smith and Bullen found a prematurity rate of 13 3 per cent in pregnant women with significant bacteriuria (100 000 bacteria or more per ml urine) and a rate of only 5 per cent in women with a negative urine culture.

In the series presented here the mothers of premature infants more often than other mothers had previously suffered from urinary infection, and they possibly also had had urinary infection more often during the current pregnancy In a special study of our series Jansson Lincoln and Winberg (1964) found significant asymptomatic bacteriuria in 13 out of 146 mothers of premature infants (9 8 per cent) and in 32 out of 664 mothers of term infants (4 8 per cent)

Whether there is a real causal connection between urinary infection and prematurity is not clear Premature birth is more common in women in bad socioeconomic conditions There also seems to be a higher rate of urinary infections in these women (Turck and Petersdorf 1962) Kass succeded in reducing the prematurity rate by adequate treatment of pregnant women with bacteriuria Similar results have recently been achieved by Rannevik (1965) who had a prematurity rate of 3 5 per cent in treated asymptomatic bacteriuria of pregnancy and 16 6 per cent in untreated cases.

As to the relationship of cigarette smoking to prematurity a considerable number of investigators have verified Simpsons (1957) original observation that pregnant women who smoke have a higher prematurity rate than those who do not. Recently Yerushalmy (1964) in an extensive prospective study found the same relationship but he was unable to confirm a higher perinatal mortality in infants of smoking mothers. On the contrary he made the surprising observation that in single live births, which fulfilled both criteria of prematurity *i e* birthweight 2500 g or less and gestational age less than 37 weeks, the neonatal mortality was significantly lower in infants of smoking than of non-smoking mothers. Yerushalmy could not explain the apparently higher survival rate for those prematures whose mothers were smoking during pregnancy

In our series we made observations of a similar kind. If the malformed infants are not included, the perinatal mortality in

premature infants of non-smoking mothers was 29 per cent, while the corresponding figure for infants of smoking mothers was 8 per cent. This can be explained by the fact that the average duration of gestation was greater in smoking than in non-smoking mothers of premature infants. Most serious obstetric complications occurred in the group of non-smokers. Thus in the absence of other complications smoking mothers seem to make a proportionately greater contribution to infants in the weight group just below 2500 g where the prognosis is better

Another observation in our series was that the number of smoking mothers of premature infants was higher in social class III than in classes I and II. It is a well established fact that premature birth is considerably more common in the lower social classes. Thus the relationship of smoking to prematurity may be indirect. As Yerushalmy points out, it may be the smoker not smoking in itself which offers an explanation of the differences observed.

Räihä, Lind, Unnérus, Kihlberg and Vara (1956) and Unnérus (1959) have, as mentioned before, shown that there exists a relationship between a small roentgenological maternal heart volume and prematurity By advising all women visiting the antenatal clinics of Helsinki, Finland, with a heart volume of 370 ml/m² body surface or less to rest lying down for some hours each day the prematurity rate was reduced from 5.5 per cent in 1954 1958 to 4.4 per cent in 1961 and the perinatal mortality from 2.6 per cent to 1 8 per cent (Räihä and Kauppinen, 1963) It is postulated that these women have a low circulatory capacity and that exercise and sedentary work with accumulation of blood in the lower part of the body cause an insufficient uterine blood flow with impairment of foetal growth. The relationship of a small heart volume to prematurity has been confirmed by Bishop in Pennsylvania, USA (1964) who found a prematurity rate of 17 1 per cent in mothers with a heart volume below 500 ml and only 7 5 per cent in mothers with a heart volume above that figure Hedberg and Rådberg (1964) in Gothenburg, however were not able to confirm these results in a small but statistically well analysed series. Terris, Gold, Schwartz and Hall (1965) criticized the previous

retrospective studies and presented a series in which the cases of prematurity and the controls were matched for comparability with respect to a number of factors known to influence the incidence of prematurity Eleven of 100 mothers of premature infants had a heart volume below 300 ml per square meter and 9 of 100 matched controls Thus no difference could be established. They concluded that possible differences in maternal heart volume are probably not independent but may reflect differences in the weight of mothers of premature and mature infants.

Raiha *et al* maintain that maternal heart volume is a good screening test to select pregnant women with an increased risk of premature birth. There are also other and perhaps simpler ways The previous discussion has dealt with several conditions having a direct or indirect relationship to prematurity If attention is payed to these states early in pregnancy close antenatal supervision can improve the results considerably as has been shown by Bishop and his collaborators (1964) There is obviously no radical solution of the problem of prematurity at least not as long as we know so little of the factors that initiate premature labour or even normal labour It is only the persistant antenatal clinical work that may lead to better results in the long term

To reduce the prematurity rate the following measures seem to be rational

1 Special attention should be given to pregnant women under 18 and over 35 years of age those who are unmarried and those who otherwise live in an unfavourable social situation. The work load of these women should be diminished as much as possible

2 Pregnant women with a previous premature birth repeated previous abortions and previous infertility should be provided with rest at home in combination with shorter or longer periods in hospital.

3 Pregnant women with hæmorrhage or imminent premature labour should be admitted to hospital. Continuous supervision in hospital can often bring the pregnancy to a happy termination.

4 All cases of toxæmia even of mild degree should be admitted to hospital Despite deficient knowledge of the ætiology

of the disease close supervision may carry the pregnancy to maturity

5. Every effort should be made to diagnose multiple pregnancy as early as possible. Bed rest may improve the results also in these cases

6 Urinary infection should be discovered and treated during pregnancy It is reasonable to investigate all pregnant women with respect to asymptomatic bacteriuria. Urinary infections have significance not only for the outcome of the present pregnancy but also and even more so for the subsequent health of the woman, as investigations in the last few years have shown. If it is impossible to perform quantitative bacterial culture in every pregnant woman at the present moment, a chemical screening test for significant bacteriuria may be an acceptable substitute.

Pregnant women should be advised not to smoke It is still uncertain whether it is the smoking in itself or some other factor pertaining to the smoking woman, that affects foetal weight, but, while waiting for further information on the influence of smoking on the growing fœtus, cigarette smoking during pregnancy must be considered as injurious.

Prophylaxis of prematurity on these lines means however that more hospital beds must be made available for antenatal care This is difficult to accomplish at the present moment when the shortage of hospital beds is a great problem in most places in Sweden. One possible way to diminish the load on the obstetric clinics is maternity nursing on self care wards as practiced by Sjöstedt (1965)

SUMMARY

A series of 223 mothers of 240 premature infants is analysed on the basis of data collected at personal retrospective interviews. The results are compared with those in 223 mothers of mature infants born during the same period (March 1 1962 February 9 1964) On the basis of the differences found and a review of the recent literature the ætiology of prematurity is discussed. Special attention is payed to the large group of cases with prematurity of unknown cause

In 45 per cent of the cases of premature birth an obvious cause such as toxæmia multiple pregnancy placental complications and fœtal malformations could be established. In the remaining 55 per cent of the cases general factors connected with prematurity such as maternal age marital status gravidity socio-economic conditions and constitution are believed to be of more importance The significance of urinary infection and smoking during pregnancy as causes of prematurity is discussed.

Some groups of pregnant women having an increased risk of premature birth are defined and measures intended to decrease the rate of prematurity and perinatal mortality are outlined.

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THE URINARY EXCRETION OF ŒSTRIOL IN POSTMATURITY

BY

FINN LUNDWALL AND GEORG STAKEMANN

The expected date for delivery is usually calculated from the date of the last period, according to the rule of Naegele. If pregnancy continues after the calculated date a state of postmaturity is supposed to be present, but there is no unanimous agreement of the exact definition of this condition In the present work the definition (of graviditas prolongata") given by Trolle (1959) has been followed, i.e a pregnancy that is more than 14 days past term (term = 280 days after the first day of the last menstrual period) provided the menstrual cycle is regular 25–35 days and the time of quickening accords with the calculation

Many American authors feel that postmaturity is unimportant, whereas most European obstetricians are of the opinion that postmaturity involves an increased risk for the baby In a careful study of postmaturity Strand (1956) concludes that perinatal mortality increases with increasing length of gestation especially in nulliparæ Lindell (1956) found that in multiparæ the perinatal mortality was unchanged (approx. 1 per cent) for gestations of 40–44 weeks duration whereas in nulliparæ the mortality increased to 6 3 per cent for gestations of over 42 weeks duration Both authors conclude that postmaturity means an increased risk to the fœtus, especially in nulliparæ

If postmaturity means a risk to the fœtus in some cases, parturition should be induced in these patients. It is difficult to

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If postmaturity means a risk to the foetus in some cases, parturition should be induced in these patients. It is difficult to

evaluate the risk in the individual case and a method to estimate the condition of the fœtus is badly needed. A definite correlation between the perinatal mortality and the oxygen tension in the fœtal blood is not unanimously agreed upon Some authors have advocated amnioscopy (Kirchhoff 1963 Zilliacus 1963)

In uncomplicated postmaturity the increased risk to the fœtus is most probably due to an ageing placenta The influence of the decreased placental function on the fœtus might be evaluated from estimations of the maternal urinary excretion of œstriol.

The excretion of this hormone in normal pregnancy has been estimated by several authors (i a Frandsen and Stakemann 1963 Coyle and Brown 1963 Green and Touchstone 1963 Klopper and Billewicz 1963 and others) In cases of fœtal death the excretion drops to very low values and it has been shown that a declining or constantly low excretion of œstriol means that the fœtus is in grave danger (Zondek and Goldberg 1957 Frandsen and Stakemann 1963 Banerjea 1962 and others) In postmaturity œstriol estimations might therefore be of value to decide if the fœtus is in danger to such a degree that pregnancy should be interrupted

Method

The method developed by Frandsen (1963) was used for all œstriol estimations and all analyses were made on 24 hour urine specimens Every effort was made to collect complete 24 hour specimens it must be emphasized however that in some cases urine may have been lost during defæcation or by mistake In such cases too low values for the hormone excretion would be obtained but major deviations can often be detected from an unusually low volume of urine

Even if complete 24 hour urine samples are collected the excretion will vary from day to day Frandsen (1963) analysed the excretion on two consecutive days in normal pregnancies and found that the excretion on the second day showed values between 60 and 140 per cent of the excretion on the first day All these factors must be taken into consideration when the results of the analyses are evaluated.

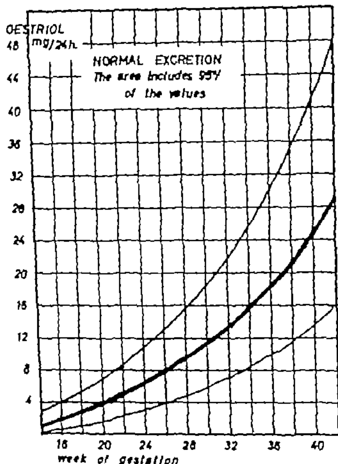


Fig The excretion of oestriol in normal pregnancy
(From Frandsen and Stakemann 963)

The excretion of oestriol in normal pregnancies is given in Fig 1 (from Frandsen and Stakemann 1963) An excretion of 16 mg/24 hrs was considered as the lower limit of the normal at the 42nd week and in the present investigation this figure is regarded as the lower limit of normal after the 42nd week also

Material

All the patients were admitted to the University Department of Obstetrics and Gynecology In this department the postmaturity rate is 10 5 per cent of all pregnancies (nulliparæ 12.2 per cent and multiparæ 8 7 per cent) The percentages of babies with a birthweight of 2500 g or less is 15 per cent, and if such babies are omitted the postmaturity rate is increased to 11 9 per cent (nulliparæ 13 7 and multiparæ 10 1 per cent)

The œstriol excretion was estimated in patients with post maturity in a two-year period (1963 and 1964) The estimations were usually started when the patient was only one week past term If a normal excretion was found the next specimen was collected one week later If abnormally low values were found, the estimations were repeated at shorter intervals

Urine was collected from 171 patients 95 of whom were nulliparæ and 76 multiparæ All cases with an abnormal obstetrical history have been excluded. In Table I the patients are grouped according to the week in which the last hormone analysis was performed

In 66 patients only one urine specimen could be collected before delivery took place (Table II) Some of these were seen for the first time when they were several weeks overdue which explains the number of single estimations even in very prolonged pregnancies

Table I *The Distribution of 171 Patients with Postmaturity Grouped According to the Week of Pregnancy at Which the Last Œstriol Analysis was Performed*

Week of Pregnancy for Last Œstriol Analyses	Nulliparæ	Multiparæ	Total
42nd	11	1	23
43rd	31	23	54
44th	28	23	51
45th	10	1	21
46th	8	5	13
47th	7	2	9
Total	95	76	171

Table II. *Number of Œstriol Analyses per Patient*

Week of pregnancy for last oestriol analysis	42nd	43rd	44th	45th	46th + 47th
Number of cases with one analysis	19	26		3	6
Number of cases with more than one analysis	4	28	39	18	16

Table III. *The Material Grouped According to the Interval Between Delivery and Last Œstriol Analysis*

Week of pregnancy for last oestriol analysis	42nd	43rd	44th	45th	46th + 47th
Less than one week between delivery and last oestriol analysis	17	52	48	17	
More than one week between delivery and last oestriol analysis	6	2	5	4	

Table III shows the time elapsing between the last oestriol analysis and delivery

Results

In Table IV the first group includes the number of patients with single normal excretions or where several estimations were within normal limits and did not show declining values. The second group consists of patients where a single value was below 16 mg/24 hrs or where consecutive estimations showed declining values (even if some or all of these were within the limits of normal excretion) The figures for both groups are broken down according to the week of the last estimation. It is interesting to observe that the percentage of pregnancies with a low or declin

Table IV *Number of Cases with Normal and With Abnormal Œstriol Excretion (Percentages in Brackets)*

Week of pregnancy for last œstriol analysis	42nd	43rd	44th	45th	46th+47th
Normal œstriol excretion	21	48	36	13	16
Low or declining œstriol excretion	2(9)	6(11)	15(29)	8(39)	6(27)

ing excretion of œstriol increased with the length of gestation the result at the 46th and 47th week being an exception for un known reasons

All 135 patients with normal excretion obtained normal, healthy babies with one exception This baby had asphyxia of second degree and died ten days later as a result of congenital heart disease

The patients with low or declining œstriol values will be considered in more detail and those with only single estimations will be described separately

A *Patients with single estimations showing excretions of less than 16 mg/24 hrs*

There were twelve patients in this group. In five cases the low values were most probably due to the urine specimen being not a complete 24 hour sample and another patient had contractions at the time when the urine was collected.

Five other women delivered completely normal babies although the œstriol excretions were only between 6 5 and 15.4 mg/24 hrs No explanation for the low excretion except postmaturity could be found.

The last patient had an excretion of 5.0 mg/24 hrs the day before she had an uncomplicated delivery of a male baby of 3400 g/52 cm However the baby had slight asphyxia.

B *Patients with several estimations showing low or declining values*

1) *Postmaturity apparently the only explanation*

Declining values were found in three cases

I Twenty-three-year-old nullipara Œstriol excretion values declined ver a week from 22 5 to 16.9 mg/24 hrs the last result was obtained in th 43rd

week. Because of the falling excretion of œstriol, labour was induced with pitocin and three days later she delivered a healthy baby of 3 50 g/5 cm.

II. seventeen-year-old nullipara. The œstriol excretion declined over a five-day-period from 17.0 to 2.4 mg/24 hrs the last result was obtained in the 45th week. Labour was induced with pitocin and she delivered three days later healthy baby of 3000 g/52 cm.

III. Nineteen-year-old nullipara. The œstriol excretion declined over a week from 18.4 to 5.8 mg/24 hrs. She delivered spontaneously five days later healthy baby of 2800 g/48 cm The placenta was circumvallate and weighed 650 g.

Constantly low values were found in four patients and in all four cases the last œstriol analysis was carried out in the 44th week:

I. Primipara In her first pregnancy she delivered baby of 2200 g two weeks befor term In the present pregnancy the œstriol excretions wer 9.7 and 6 mg/24 hrs Four days later she delivered spontaneously a healthy baby of 2500 g/49 cm

II Primipara Excretion of œstriol was 1 3- 3 3 and 3. mg/24 hrs Three days later she delivered spontaneously a healthy baby of 3750 g/53 cm.

III Seventeen-year-old nullipara. Excretion of œstriol 3.4-9 6 and 3 6 mg/24 hrs. Labour was induced with pitocin and the next day she delivered a healthy baby of 3250 g/49 cm.

IV Eighteen-year-old nullipara Excretion of œstriol 3.2, 14.3 and 3 8 mg/24 hrs Labour as induced with pitocin and two days later she delivered healthy baby of 3000 g/51 cm.

) *Urine pecimen probably not complete*

I. Thirty-year-old primipara Fig. illustrates the declining values of the œstriol output in the 45th week, but the volume of urine diminished at the same rate Although the patient was in hospital all the time, some urine must have been spilled when the last two samples were collected She delivered later healthy baby of 2950 g/50 cm. The placenta was normal.

3) *Discoloured amniotic fluid*

I Twentytwo-year-old nullipara The œstriol excretion is given in Fig 3 and the last result was obtained at the end of the *44th* week. At the time when the report of an excretion of only 5 mg/24 hrs (50 per cent of the two previous ations) arrived, a new sample of urine had been collected However it as felt that the great decline in hormone excretion made it necessary deliver the baby at once and Cæsarean section was carried out. The amniotic fluid showed greenish discolouration but healthy baby of 3500 g cm was delivered The placenta was normal. The urine specimen ollected just before the Cæsarean section showed an excretion of 21 5 mg 24 hrs. The low excretion of œstriol and the discoloured amniotic fluid may be explained by transitory stress ituation of the fœtus.

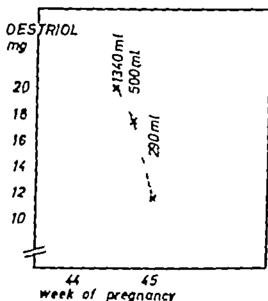


Fig 2. Urinary oestriol excretion in a thirty-year-old primipara. The decreasing values are most probable due to incomplete 24 hrs urine samples. Case report on page 307

If amnioscopy had been carried out in this patient immediate delivery would have been indicated according to the criteria given by *Kirchoff* (1963) and *Zilliacus* (1963) This patient is the only case in the present series where discoloured amniotic fluid was found.

4) Uterine contractions when the urine was collected

In six patients declining values were found but uterine contractions were present at the time of the low excretion.

I Nullipara. Œstriol excretion declined from 19.0 to 13.4 mg/24 hrs the last result was obtained in the 45th week. She delivered a healthy baby of 3150 g/50 cm.

II. Nullipara. Œstriol excretion declined from 21.2 to 14.6 mg/24 hrs the last result was obtained in the 45th week She delivered a healthy baby of 3350 g/54 cm.

III. Nullipara. The oestriol excretion declined from 16.5 to 8.2 mg/24 hrs the last result was obtained in the 45th week. She delivered a healthy baby of only 2350 g/48 cm.

IV Secondpara. The oestriol excretion declined from 21.0 mg to 2.2 mg/24 hrs the last result was obtained in the 45th week She delivered a healthy baby of 3400 g/52 cm.

V Nullipara. The oestriol excretion declined from 22.4 to 7.0 mg/24 hrs

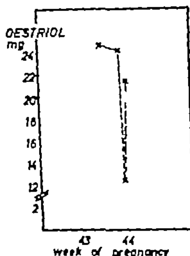


Fig. 3 The urinary excretion of œstriol in twenty-year-old nullipara. At delivery greenish discolouration of the amniotic fluid was found.
Case report on page 307

the last result was obtained in the 46th week. She delivered healthy baby of 3200 g/5 cm.

VI. Nullipara. The œstriol excretion declined from 20. to 0.9 mg/24 hrs, the last result was obtained in the 47th week. She delivered a healthy baby of 3550 g/54 cm.

A combination of uterine contractions and a low excretion of œstriol has been demonstrated previously (Frandsen and Stakemann 1963) and it cannot be excluded that this coincidence is not accidental. Clinically the low excretion in these cases is not of great importance as in most cases delivery will have taken place before the result of the hormone analysis is available

5) Infarcts of the placenta

In only 9 of the 17 patients were placental infarcts demonstrated, and in five cases the infarcts were very small (less than 1/ ccm per placenta)

One of the other four women had only single œstriol estimation, performed one week before delivery and it showed normal excretion The fœtal heart rate diminished immediately befor delivery but a healthy baby

was delivered

A second patient had three estimations over a 7-day period the last result was obtained in the 44th week. Values of 16.1–12.5 and 12.6 mg/24 hrs were found. The day after the last urine sample had been collected, she delivered spontaneously a healthy baby of only 2750 g/51 cm. The placenta contained eight infarcts of approximately 1 ccm each

The third patient was a 32-year-old nullipara. The oestriol excretion had been estimated regularly in the 41st–45th week, and a total of 12 estimations had been carried out. At first a declining excretion was found (18.2–16.4–13 3 mg/24 hrs) then the output rose to approximately 20 mg/24 hrs and was stabilized at this level for some time. Then a new decline followed by another increase took place (21.6–16.8–17.4–28.6 mg/24 hrs) Labour was induced in the 45th week by pitocin. She delivered a healthy baby of ~200 g/51 cm. The placenta contained a 3×3 cm infarct.

The fourth patient delivered a stillborn baby and this case will be described later (under section 8)

Infarcts of the placenta are an uncommon occurrence in cases of postmaturity As judged from the amounts of oestriol excreted it seems that the placenta can regain its ability to nourish the foetus properly when the acute phase of placental degeneration is over In this connection another patient, not included in the present series should be mentioned. Clinically the pregnancy was completely normal but the oestriol excretion was declining constantly (Fig 4) Induction of labour with pitocin was tried but was unsuccessful Because of the low hormone output a Caesarean section was performed two weeks after term and a healthy baby of 3150 g/50 cm was delivered The placenta contained four big, white infarcts It must be supposed that the life of this foetus was in grave danger The clinical condition gave no hint of this danger which was only diagnosed from the low hormone excretions

6) Cases with slow foetal heart rates at delivery

In three patients the foetal heart rate slowed during delivery All women had had a diminishing oestriol excretion in the period preceding delivery

I. Primipara. The oestriol excretion declined from 2 7 to 15 3 mg/24 hrs the last result was obtained in the 44th week. Just before spontaneous delivery the foetal heart rate slowed down. The baby was asphyxiated but recovered in a short time The size was 3500 g/52 cm.

II. Nullipara. The oestriol excretion declined from 25 7 to 18.0 mg/24 hrs the last result was obtained in the 44th week. She went into labour the next

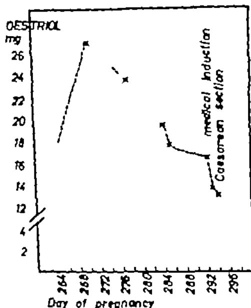


Fig. 4 Urinary excretion of oestriol in a patient with placental infarcts
Case report on page 3

day At an early phase in labour the foetal heart sounds slowed and an immediate Caesarean section was carried out. A healthy baby of 3650 g/54 cm was obtained

III Nullipara The oestriol excretion declined from 7.8 to 5.6 mg/24 hrs the last result was obtained in the 47th week Caesarean section had to be performed because of a slow foetal heart rate during spontaneous labour A healthy baby of 3300 g/52 cm was obtained

In all three cases the placenta was normal and no reason for the low foetal heart rate could be found.

7) Dysmaturity

Unfortunately the records give only meagre information about signs or symptoms of dysmaturity of the babies Three babies are explicitly described as dysmature In these cases declining values for the oestriol excretion were found.

I Nullipara 21 years old. The oestriol excretion declined from 9.8 to 5.4 mg/24 hrs, the last result was obtained in the 43rd week Two days later she delivered spontaneously healthy baby of 3000 g/50 cm The placenta was normal.

II. Twentytwo-year-old nullipara. The oestriol excretion declined from 15.2 to 13.1 mg/24 hrs, the last result was obtained in the 44th week. Two days later she delivered spontaneously a healthy baby of 2900 g/53 cm.

III. Primipara. The oestriol excretion declined in the last two weeks preceding delivery from 19.9–17.6 to 14.6 mg/24 hrs, the last result was obtained in the 44th week. Four days later she delivered spontaneously a healthy baby of 2700 g/52 cm.

B) *Deliveries of stillborn babies*

I. Thirtytwo-year-old primigravida. Expected date of delivery: June 20th. The excretion of oestriol was as follows: June 11th 14.1, July 15th 8.5, July 16th 4.3 mg/24 hrs. Before the last result was obtained, the heart sounds stopped on July 17th. At this time uterine contractions were present. The membranes were ruptured and pitocin given. A stillborn female baby of 3800 g/53 cm without malformations was born. The placenta weighed 700 g and contained two infarcts of 3×3 cm.

This patient was one of the first seen at the time when the importance of oestriol estimations was realized in the Department. With our present knowledge labour would have been induced when the report of the first low oestriol excretion had arrived.

II. Thirtynine-year-old nullipara. She was first seen in the outpatient ward when she was at the 45th week of gestation. She was asked to collect a 24 hour urine specimen. Clinically everything was normal. Serious illness followed by the death of her mother made her go to another town. Thus it was impossible to contact her when the result of the oestriol analysis was available. It showed an excretion of only 7.4 mg/24 hrs. She was first seen five days later. Foetal heart sounds could no longer be heard. A new urine sample showed an excretion of only 0.8 mg/24 hrs. Three days still later she delivered a dead, macerated baby.

The normal excretion of œstriol after 42 weeks of gestation

In the literature little information is available concerning the excretion of oestriol after 41 weeks of gestation. Furuhjelm (196-) found in 22 cases of postmaturity an average excretion of oestriol of 13.9 ± 7.5 mg/24 hrs. 7 patients excreted less than 9 mg/24 hrs. There was a tendency to lower excretions with longer gestations, but the correlation was not statistically significant.

Fig. 5 shows the oestriol excretion values obtained after 41 weeks gestation in the present series. Excluded are cases where an incomplete 24 hour sample was suspected, cases with uterine contractions at the time of urine collection and cases with placental infarcts. In this way it is believed that Fig. 5 shows the

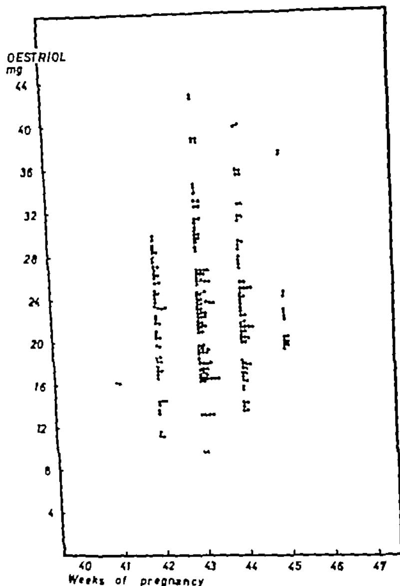


Fig. 5. The urinary excretion of œstriol after the 4 t week of gestation. All patients delivered after the 42nd week. If several similar values were found at the same gestational week, they are presented in a row. All values found in the present series are given except such where the urine sample was incomplete or uterine contractions were present when the urine was collected. For details see page 3 2.

œstriol excretions in cases of prolonged but otherwise normal pregnancies It is seen that the values are widely scattered around an average of 24–25 mg/24 hrs but with no tendency to lower values with longer gestation periods

Fig 6 shows the correlation between the œstriol excretion in the last week preceding delivery and the weight of the baby It seems reasonable to believe that a positive correlation exists between the two parameters.

The results in Figs 5 and 6 mean that there is no relation between the weight of the baby and the length of gestation. Fig 7 shows that this is correct

The excretion of œstriol in cases of postmaturity in nulliparæ and multiparæ

Table I shows that the distribution of nulliparæ and multiparæ in relation to the time of the last œstriol estimation is similar Table II shows that a total of 37 patients had a low or decreasing excretion of œstriol Of these patients six are excluded because the urine specimens most probably were not complete 24-hour samples and seven because they had contractions when the urine was collected. The distribution of the other 24 cases is shown in Table V The result shows that 19 (20 per cent) of the nulliparæ had a low or decreasing excretion of œstriol, while the figure for multiparæ is only 5 (6 per cent) All still births occurred in nulliparæ The figures demonstrate that post maturity means an especially increased risk for nulliparæ

Conclusions

Although the definition of postmaturity is very explicit, such patients make up a very mixed, unhomogeneous group The diag nosis is based on patient information only and other factors too can make the calculations uncertain There is no doubt that in many cases of postmaturity the pregnancy is not prolonged but the date of confinement miscalculated

In the present series the definition of Trolle (1959) in Nomenclatura obstetrica has been used. Cases were only omitted if

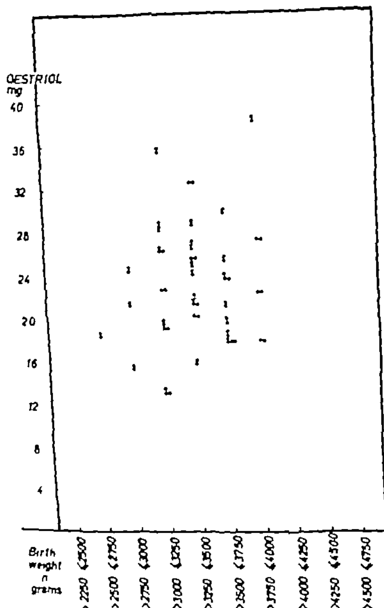


Fig. 6. The urinary excretion of oestriol in the week preceding postmature delivery compared with the birth weight of the infant

œstriol excretions in cases of prolonged but otherwise normal pregnancies. It is seen that the values are widely scattered around an average of 24–25 mg/24 hrs but with no tendency to lower values with longer gestation periods

Fig 6 shows the correlation between the œstriol excretion in the last week preceding delivery and the weight of the baby It seems reasonable to believe that a positive correlation exists between the two parameters.

The results in Figs. 5 and 6 mean that there is no relation between the weight of the baby and the length of gestation. Fig 7 shows that this is correct.

The excretion of œstriol in cases of postmaturity in nulliparæ and multiparæ

Table I shows that the distribution of nulliparæ and multiparæ in relation to the time of the last œstriol estimation is similar Table II shows that a total of 37 patients had a low or decreasing excretion of œstriol Of these patients six are excluded because the urine specimens most probably were not complete 24-hour samples and seven because they had contractions when the urine was collected The distribution of the other 24 cases is shown in Table V The result shows that 19 (20 per cent) of the nulliparæ had a low or decreasing excretion of œstriol, while the figure for multiparæ is only 5 (6 per cent) All still births occurred in nulliparæ The figures demonstrate that post maturity means an especially increased risk for nulliparæ

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In the present series the definition of Trolle (1959) in "Nomenclatura obstetrica has been used Cases were only omitted if

Table V Number of Cases With Low or Declining Excretion of Œstriol Grouped According t the Clinical Conditions

		Nullipara	Multipara
Number of cases with one oestriol analysis	Postmaturity apparently the only explanation	5	
	Infant condition poor at delivery		
Number of cases with more than one oestriol analysis	Postmaturity apparently the only explanation	5	I
	Discoloured amniotic fluid	I	
	Placental infarcts		
	Slow foetal heart rate at delivery		
	Dysmaturity		
	Stillborn infant		
Total		9	5

One also had placental infarcts

menstruation was irregular or the time of quickening was not in agreement with the other information

Fig 5 shows that the excretion of oestriol does not increase after the 42nd week of gestation If postmaturity is a pathological condition one cannot speak of normal excretion values after the 42nd week As 16 mg/24hrs is the lowest excretion we have seen in normal pregnancies of 40–42 weeks of gestation (Frandsen and Stakemann 1963) we have considered excretion levels beneath this value as decreased also in postmaturity This limit seems reasonable since all patients with higher excretion values in the present investigation had normal pregnancies and delivered normal babies of which only one died later (from congenital heart disease on the 10th day)

In cases with excretion levels of less than 16 mg/24 hrs. signs of a foetal stress situation were not evident in every case but a large proportion did show such findings. Of the 37 patients

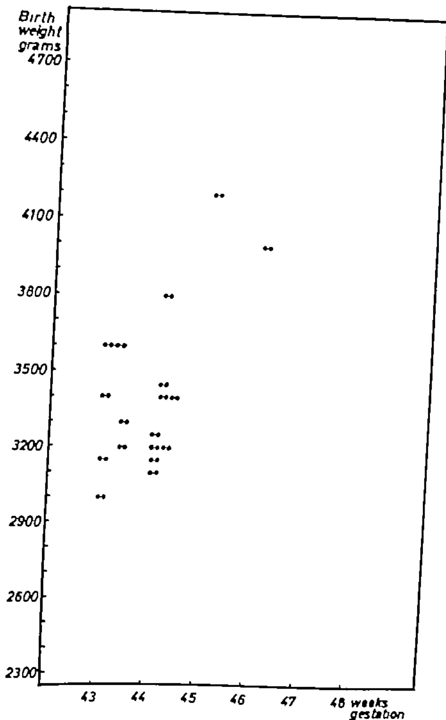


Fig. 7 The birth weight of the infants in relation to duration of gestation.

foetus, but at the present time the above mentioned policy is followed in our department. It is hoped that these suggestions will be proved justified when a larger series has been collected.

SUMMARY

The urinary excretion of oestriol after 42 weeks gestation has been investigated in 171 women. Ninety-five were nulliparæ and 76 were multiparæ. Low excretion values (less than 16 mg/24 hrs) were found in 37 patients. Of these a foetal stress situation could be suspected in 24 cases and symptoms to this effect was demonstrated in twelve. Two of these babies died. Low excretions were more frequent among nulliparæ (19 cases or 20 per cent) than among multiparæ (5 cases, or 6 per cent) In the 95 cases with a normal excretion of oestriol no signs of foetal stress or perinatal deaths were found.

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with a low or decreasing excretion the low hormone values were in thirteen cases most probable due to incomplete 24 hrs urine samples or uterine contractions at the time of the urine collection This leaves 24 cases where a foetal stress situation could be suspected from the low œstriol output. In twelve of these some pathological features to this effect was found and the only two stillborn babies occurred in this group No abnormal features were found among the 132 patients with an excretion of more than 16 mg/24 hrs.

In the other twelve cases with low excretion values the only abnormal condition found was postmaturity

In other words pathological conditions in addition to postmaturity were present in 50 per cent of cases with a low or decreasing excretion of œstriol If 12 mg/24 hrs rather than 16 mg, is taken as the lower limit of normal the proportion of cases with additional abnormal features did not increase

Others have demonstrated that postmaturity involves a greater risk to nulliparæ than to multiparæ In accordance with this result both our stillbirths occurred in nulliparæ Even more striking is our finding that decreased œstriol excretions were found in 20 per cent of nulliparæ but only in 6 per cent of multiparæ with postmaturity

The policy of obstetricians in cases of postmaturity varies but many will let the pregnancy continue after the 42nd week at least if the foetal weight is estimated as less than 2500 g This policy undoubtedly means an increased risk to the fœtus in some cases and it seems that these cases can be picked out by serial œstriol estimations If the hormone excretion is not decreasing and is over 16 mg/24 hrs the pregnancy should be allowed to continue the œstriol excretion being estimated at intervals. If a decreasing excretion or values below 16 mg/24 hrs are encountered the whole situation should be evaluated most carefully

In our opinion the pregnancy should be interrupted in most such cases

In patients with severely impaired œstriol excretion an immediate Cæsarean section should be performed otherwise labour is induced artificially It is not quite clear whether induction of labour in cases of postmaturity involves a special risk to the

and Pritchard (1961) considered the variation great in some subjects, but in their series there were also women with only a small variation between the periods.

Hallberg and Nilsson (1964 b) studied 12 student nurses and made consecutive measurements of 12 periods An analysis of variance showed that the variation between subjects was great and statistically significant, and that the variation in the individual subjects was small and not statistically significant. Thus, it was concluded that a few determinations or even a single deter mination of the menstrual blood loss fairly well characterises the average individual blood loss

On the basis of this conclusion Hallberg, Högdahl, Nilsson and Rybo (1966) studied 137 industrial female workers. In 117 of the women the menstrual blood loss was measured during two consecutive periods. There was a good agreement between the two periods in the same subject. In this study it was also found that only 7 per cent of the subjects had a haemoglobin concentration below 12 g/100 ml blood, a figure which is considerably lower than that reported by others (Kilpatrick 1961 Kilpatrick and Hardisty 1961 Jacobs, Kilpat rick and Withey 1965) The menstrual blood loss is probably a major factor influencing the iron balance in women. Thus the finding of an unexpectedly low frequency of iron deficiency in the series of female industrial workers may indicate that the observed menstrual blood loss is not representative for the population as a whole.

This assumption and the fact that the subjects in previous studies had not been selected at random initiated the present study of a large series selected at random from a population.

Such a study is necessary in order to get adequate information on the variation of the menstrual iron loss in women and to try to establish the normal loss in a population. Knowledge of the magnitude of the normal menstrual blood loss is often necessary in gynaecological and medical practice. In a large series the limits of normality can be evaluated not only by applying arious statistical methods but also by recording the presence and frequency of iron deficiency subjecti e complaints etc.

Thus the main purpose of the present study was

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MENSTRUAL BLOOD LOSS—A POPULATION STUDY

Variation at different ages and attempts to define normality

BY

LEIF HALLBERG, ANN-MARIE HÖGDAHL, LENNART NILSSON AND GÖRAN RYBO

Studies of the menstrual blood loss have been performed before by several authors. Usually these investigations have been based on small and selected series involving hospital personnel or students. Early reports on menstrual blood loss were reviewed by Barer and Fowler (1936) and also by Frenchman and Johnston (1949). A summary of the reports after 1936 is given in Table I. The table shows that the series studied differed in many respects and that various technical procedures were used to determine the menstrual blood loss.

Repeated measurements of consecutive periods were made in some investigations and a good agreement between the various periods in the same individual has been reported by most authors (Barer and Fowler 1936, Leverton and Roberts 1937, Frenchman and Johnston 1949, Millis 1951, Apte and Venkatachalam 1963 and Hytten, Cheyne and Klopper 1964). Other authors, however, have found a great variation between various periods in the same individual. Thus Arens (1945) found that the menstrual blood loss varied from one period to another in 5 out of 7 women whose menstrual blood loss was measured more than once. Schlapphoff and Johnston (1949) also reported great variation between different periods in 6 girls aged 13 to 14 years. Baldwin, Whalley

Rankin V all, Huntsman, and Liddell England 962	20		—	Radio isotope technique (Cr^{51})	—	9 3—870 ml blood	—	Patients complaining of menorrhagia
Apte and Venkatachalam, India 963	3		—	Chemical determination of iron	37 ml blood	10—63 ml blood	—6 in subjects	Healthy adult non-anemic females
Gültner and Geller Germany 964	20	33 (mean value)	7	Electrical conductivity measurements	69.3 ml blood	— ml blood	—	Healthy women who considered their menstruation normal
Hallberg and Nilsson Sweden 964			3	Hemoglobin determination	28 ml blood	9—55 8 ml blood		Healthy non-anemic nursing students
Hytten, Cheyne and Klopper Scotland 964	38		2—38	Chemical determination of iron	25 ml blood	7—70 ml blood	—4	15 healthy nulliparous midwives and 3 healthy parous women
Price Forsyth Cohn, and Cronkite USA 964	7		4—46	Radio isotope technique (Fe^{59}) Whole-body counting	—	33—592 ml blood	2—4	51 women complained of menorrhagia on considered her menstruation normal
Jacobs and Butler England 965	7		—40	Radio isotope technique (Cr^{51})	34 7 ml blood	3—87 ml blood	—	Healthy midwives with a hemoglobin concentration not less than g/100 ml In series of 14 subjects with iron deficiency the mean value was 85 5 ml blood
Hallberg, Hogdahl, Nilsson, and Rybo Sweden, 1966	37		6—52	Hemoglobin determination	34 ml blood	1.6—90.7 ml blood	2 in 7 subjects	Industrial workers

Table I *Results Obtained in Previous Studies on Menstrual Blood Loss*

Author	Number of Subjects	Age (Range)	Method of Estimating Blood Loss	Blood Loss Mean	Blood Loss Range	Number of Periods in Subject Measured More than Once	Remarks
Barer and Fowler USA 1936	100	15—43	Chemical determination of iron	50 55 ml blood	6 55—178 69 ml blood	2—4 in 13 subjects	Hospital employees who considered their menstruation normal Hæmoglobin above 10.2 g/100 ml blood
Leverton and Roberts USA 1937	4	21—27	Chemical determination of iron	34.24 ml blood	26.48—50 78 ml blood	3—6	Students
Moore Minnich and Welch USA 1939	14	—	Hæmoglobin determination	—	9—41 ml blood	—	Young college women
Arens USA 1945	51	5—23	Chemical determination of iron	58.9 ml blood	11 7—157.8 ml blood	2—3 in 7 subjects	Hæmoglobin above 10.4 g/100 ml blood.
Schlapphoff and Johnston USA 1949	6	13—14	Chemical determination of iron	17 62 mg iron	6 12—50 11 mg iron	4—6	Healthy subjects with good dietary habits
Millis Australia 1951	14	21—27	Chemical determination of iron	21 8 mg iron	3 5—66.8 mg iron	3—5	Healthy nursing students who considered their menstruation normal
Baldwin Whalley and Pritchard USA 1961	21	—	Radio isotope technique (Fe^{59})	25 ml blood	10—55 ml blood	2—3 in 18 subjects	Healthy nurses nursing students and technicians who considered their menstruation normal
Hagedorn Kiely Tauxe and Owen USA 96	12	—	Radi isotope technique (Cr^{51})	27 ml blood	6—50 ml blood	—	Healthy young women. In eries of 5 iron deficient subject the blood los range was —379 ml

Rankin, Veall Huntsman and Liddell England 962	30	—	Radio isotope technique (Cr[51])	—	0 3—970.0 ml blood	—	Patients complaining of menorrhagia
Apte and Venkatachalam India 963	3	—	Chemical determination of iron	37 ml blood	10—62 ml blood	—6 in 12 subjects	Healthy adult non-anemic females
Goltner and Gaffer Germany 964	30	33 ± 7 (mean value)	Electrical conductivity measurements	69 3 ml blood	— ml blood	—	Healthy women who considered their menstruation normal
Hallberg and Nilsson, Sweden 964		— 3	Hemoglobin determination	28 ml blood	9 —55 8 ml blood	2	Healthy non-anemic nursing students
Hytten, Cheyne, and Klopper Scotland 964	38	—38	Chemical determination of iron	25 ml blood	7—70 ml blood	2—4	5 healthy nulliparous midwives and 3 healthy parous women
Price Forsyth, Cohn, and Cronkite USA 964	7	4—46	Radio isotope technique (F [59]) Whole-body counting	—	33—352 ml blood	2—4	Six women complained of menorrhagia, one considered her menstruation normal
Jacobs and Butler England 965	7	—40	Radio isotope technique (Cr[51])	34 7 ml blood	3—87 ml blood	—	Healthy midwives with a hemoglobin concentration not less than g/100 ml. In a series of 4 subjects with iron deficiency the mean value was 83 5 ml blood
Hallberg, Högdahl Nilsson, and Rybo Sweden, 966	37	6—52	Hemoglobin determination	34 ml blood	6— 99.7 ml blood	In 7 subjects	Industrial workers

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Baldwin Whalley and Pritchard USA 1961	21	—	Radio isotope technique (Fe^{59})	25 ml blood	10—55 ml blood	2—3 in 8 subjects	Healthy nurses nursing students and technicians who considered thei menstruation normal
Hagedorn Kiely Twuse and Owen USA 96		—	Radio isotope technique (Cr^{51})	27 ml blood	6—50 ml blood	—	Healthy young women. In a series of 5 1 on deficient subjects th blood loss range was 3—574 ml

the number born on that day was insufficient, further subjects were selected from women born on 13th March, 15th March etc. until 125 subjects were obtained.

A letter explaining the purpose of the study was sent to all subjects. In the letter it was also pointed out that they were offered a free health screening. Later they were called up or contacted through a new letter and an appointment was arranged for the examination

The reason for selecting the above-mentioned ages was At 15 years of age in most girls the menarche has occurred recently and usually such girls have not been pregnant. Furthermore, the rapid phase of growth has ceased in most subjects. The age of 23 was chosen because at this age half of the women are married (Statistical year book of Göteborg, 1962) and many have also born children. At the age of 30, the birth frequency is near maximum. At the age of 40 the birth frequency has decreased markedly Nearly all the 40-year-old women are still menstruating and it is not likely that any climacteric influences have occurred. The 45 and 50-year groups were selected to study the time period near the menopause

Due to recent change of name by marriage, two women at the age of 45 were falsely registered twice Consequently this group actually comprised only 123 women.

3. *Withdrawals from the trial*

The number of selected subjects who did not participate in the examination is given in Table II, which shows that 85 women (11 3 per cent of the selected subjects) did not take part in the investigation. Out of these however 31 (4.1 per cent of the selected women) had moved from the city recently and their names had not been removed from the census register 54 women (7 5 per cent of the remaining subjects) did not participate for reasons given in Table III.

Of the women taking part in the study 152 subjects (22.9 per cent) had amenorrhoea (Table IV) The causes of the amenorrhoea are given in Table V Thus as is shown in Table VI there remained 511 menstruating women. Of these however data of

1 to study the variation of the menstrual blood loss at various ages in a large series selected at random.
2 to try to establish the upper normal limit of the menstrual blood loss

MATERIAL AND METHODS

1 *Outline of the study*

The present study was made in 476 women selected at random by stratified sampling from the population of Göteborg. Women of six ages were studied and in each group 125 subjects were selected.

The study was designed as a health screening programme and was part of a more extensive population study Besides a gynæcological history with detailed recording of the menstrual pattern, the study included a social a dietary and a general medical past history Height weight certain skinfolds and blood pressure were measured Blood samples were drawn to determine hæmoglobin concentration hæmatocrit and plasma iron concentration Urine was tested for albumin and sugar Other analyses of blood and urine were also made and will be reported separately The measurement of the menstrual blood loss was usually performed during the period immediately after the examination.

Most of the subjects were examined between April and June 1963 but 46 women who for various reasons could not be reached in 1963 were examined between September and December 1964

2 *Material*

The subjects were selected from the following six age strata 15 23 30 40 45 and 50 years In each age group women born on 14th March were selected until 125 subjects were obtained. If

Hormone preparations with an ovulation-inhibiting effect are known to influence the menstrual blood loss However such preparations were not approved for contraceptive purposes in Sweden in the spring of 1963. These tablets were therefore rarely used. Furthermore all women were asked if they took any tablets or other medicines. Only two women stated that they were using ovulation-inhibiting hormone-preparations

Table IV *Number of Examined Subjects at Different Ages with Amenorrhoea*

Age	5	25	30	40	45	50	Total
Examined subjects	1 4	108	113	06	09	113	663
Examined subjects with amenorrhoea							
Number		20	19	10	8	73	152
Per cent	1 5	18 5	16 8	9.4	16 5	64 6	22 9
Examined subjects with menstruation							
Number	02	88	94	96	9	40	511
Per cent	69 5	81 5	83 2	90.6	83 5	35 4	77

Table V Causes of *Amenorrhoea*

Age	15	25	30	40	45	50	Total	Per Cent of Subjects with Amenorrhoea
"Delayed menarche"	8	—	—	—	—	—	8	5 3
Pregnancy		8	14	—	—	—	33	7
Lactating	—		3	—	—	—	5	3 3
Surgery[1]	—	—	—	6	5	9	20	3
Climacteric	—	—	—		3	64	79	52.0
Other causes of amenorrhoea ("Secondary amenorrhoea")	3	—		2	—	—	7	4 6
Total		20	9	0	8	73	52	00 1

Hysterectomy and/or oophorectomy In one case amenorrhoea resulted from irradiation treatment

4 Methods

The determination of the menstrual blood loss was performed by the method of Hallberg and Nilsson (1964 a) Briefly the procedure was as follows The women were carefully instructed by a nurse how to collect their menstrual blood. It was emphasized that waste of blood must be avoided. The women were asked to use if possible both a sanitary towel and a tampon at the same time in order to avoid waste of blood. When visiting the toilet they were requested to have a tampon in their vagina.

Table II. *Number of Selected and Examined Subjects at Different Ages*

Age	15	20	30	40	45	50	Total	Per Cent of Selected Subjects	Per Cent of Remaining Subjects
Selected subjects	125	125	125	125	123	125	748	—	—
Moved from the city	3	11	5	9	2	1	31	4.1	—
Remaining	122	114	120	116	121	124	717	95.9	—
Non-response	8	6	7	10	12	11	54	7.2	7.5
Examined subjects	114	108	113	106	109	113	663	88.6	92.5

Table III. *Reasons Why Women Who Lived in the City Did Not Participate in the Examination*

Age	15		30	40	45	50	Total
Not available	1	1	2	2	—	—	6
Refusal without motive	—	—	—	1	2	1	4
Lack of time	4	2	4	5	6	7	8
Recent medical examination	2	1	—	—	1		5
Mental illness	1	1	—	2	3	1	8
Severe alcoholism	—	1	1	—	—	—	2
Dead prior to examination	—	—	—	—	—	1	1
Total	8	6	7	10	12	11	54

Usually due to employment, custody of children or nursing sick relatives.

Judging from the subjects own statements one 15-year-old girl had anaemia and one 50-year-old woman had recently been examined at a radiologic clinic due to malignant disease. The others were healthy.

Two women were living in a nursing home due to mental debility three were in a mental hospital and five were out patients in a psychiatric clinic.

Cause of death. Cardiac insufficiency due to aortic stenosis.

the menstrual blood loss was not obtained in 35 (6.8 per cent) for reasons given in Table VII.

The series in which the menstrual blood loss was determined thus included 476 subjects. The number of subjects in each age-group is given in Table VI.

mined as cyanmethæmoglobin. The hæmatocrit was determined using a microhæmatocrit centrifuge (International) The mean corpuscular hæmoglobin concentration (MCHC) was estimated without correction for trapped plasma

The data was coded for automatic data processing A special programme was worked out for the analyses.

Conventional methods were used for the statistical significance tests calculation of the mean and median values and the standard error of the mean (Hald, 1951) When two or more tests are not independent the significance levels were corrected in order to ensure that the significance of the combined tests was at least on the five per cent level.

RESULTS

The entire series was first studied. Statistical methods were used to try to define the limits of the normal menstrual blood loss. Then certain subjects, fulfilling various criteria of normality were studied. Finally attempts were made to define the upper limit of the normal menstrual blood loss on the basis of the frequency of subjects with criteria of iron deficiency in different ranges of menstrual blood loss.

1 *Menstrual blood loss of the entire series*

Table VIII shows the mean values of the menstrual blood loss for each age group as well as for the entire series At the age of 50 the mean value was higher than at the other ages but the differences were not statistically significant. However the variance in the 50-year group was statistically higher than in the group 23–45 years ($F = 2.489$ df $= 37$ and 344 $p < 0.01$)

The menstrual blood loss in the 15-year-group was lower than in the others. The difference between the mean value of this group and the mean value of the subjects aged 23–45 years was statistically significant ($z = 2.800$ $p < 0.01$)

One reason for the difference observed might be a difference in collection technique between the 15 year-group and the other

The data processing connected with this project was performed on the Facit EDB 3 computer Industridata AB Göteborg

Table VI. *Number of Women at Different Ages in Whom Menstrual Blood Loss Was Measured*

Age	5	3	30	40	45	50	Total
Menstruating women	102	88	94	96	91	40	511
Women with measured blood loss							
Number	95	77	89	92	86	37	476
Per cent of menstruating women	93 1	87 5	94.7	95 8	94.5	92.5	93 2
Women with blood loss not measured							
Number	7	11	5	4	5	3	35
Per cent of menstruating women	6.9	12 5	5.3	4.2	5 5	7 5	6.8

Table VII. *Reasons for Missing Menstrual Blood Loss Data*

Age	5	3	30	40	45	50	Total
Refusal to participate due to discomfort	4	3	—	1	4	2	14
Subject not instructed	1	1	—	2	—	—	4
Container not returned	2	6	2	—	1	1	12
Containers lost	—	1	3	1	—	—	5
Total	7	11	5	4	5	3	35

The sanitary towels and tampons used were collected at home in a plastic container with a tight cover

When the containers were brought to the laboratory the blood in the towels and tampons was extracted with a 5 per cent NaOH solution, thus transforming the hæmoglobin into alkaline hæmatin, which was estimated spectrophotometrically The amount of the menstrual hæmoglobin determined was expressed as millilitres blood using the hæmoglobin concentration in the venous blood

Even if attempts are made to avoid any waste of blood during the collection, such might occur The values obtained therefore must be regarded as minimum figures of the menstrual blood loss

Plasma iron concentration was determined by the method of Bothwell and Mallet (1955) and the hæmoglobin was deter

Table VIII *Mean Median and Certain Percentile Values of Menstrual Blood Loss in ml in Different Age-Groups*

Age			30	40	45	50	Total
Number of subjects	65	77	89	[illegible]	86	37	476
Mean value ± standard error of mean	33.8 4	38.9±3.7	40 ±7.0	44.5±5.7	47.7±4.5	62.4±13.2	43.4± 3
10th percentile	4	8.7		0	7.9	13	0.4
25th percentile	9.3	16.9	5	20	6.7	9.3	8.2
Median value	25.4	30.6	30.9	30.8	29.5	36.4	30.0
75th percentile	44.5	52.6	57.3	48.3	54.5	69.5	52.4
90th percentile	65	77.8	88.3	87	89	133.1	83.9

Table IX. *Mean Value of Menstrual Blood Loss in Different Age-Groups for Subjects Using Both Tampons and Towels and for Subjects Using Only Towels*

Age			30	40	45	50	Total
Number of subjects using tampons and towels	44	70	84	70	69	20	357
Mean value ± standard error of mean	36 3.5	39.4±3.0	48 ±7	45.5±7	43.9±5.0	6 .2±22.1	44.7±2.0
Number of subjects using only towels	51	7	5		7	17	1 9
Mean value ± standard error of mean	32.3±3	36.4±7.7	53 ±27.7	4 .4±6.3	37.9±9.7	63.8±13.3	40.8±3.2

groups. Therefore, a study was made of the actual use of towels and tampons in all age-groups As shown in Table IX lower mean values were obtained for women using only towels in four of the age-groups (15, 23 40 and 45 years) In the 30- and 50-year groups the mean values were lower for women who used towels and tampons than for those who used towels only However the value is based on comparatively few subjects in the 30-year group In the 50-year group furthermore the mean value was greatly influenced by some extreme values. When the mean values of those in the 15 year group *using tampons and towels are compared with the corresponding value in the age-*group 23–45 years no significant difference was found.

The distribution of women in relation to the menstrual blood loss is shown in Fig 1 and of women in the various age-groups in Fig 2. The median values were rather similar at all ages except in the women aged 50 years whose median value was higher than at the other ages. In Table VIII the values of the 10th 25th 75th and 90th percentiles are reported. The 90th percentile values are also shown in Fig. 2 The 75th and 90th percentile values too were higher in the 50-year-group than in the other groups

II *Menstrual blood loss in healthy women*

In an attempt to define the limits of normality of the menstrual blood loss a study was made of subjects fullfilling certain criteria of normality Two series were studied.

In one series (Series A) the assessment of normality was based only on the subjects own statements This series thus included only those who at the time of the examination, considered themselves quite healthy and capable of working and who moreover considered their menstruation normal This series comprised 357 subjects

In another series (Series B) further criteria of normality were applied. As heavy menstrual blood loss may result in iron deficiency attempts were made to reduce the number of iron deficient subjects Therefore besides the criteria of normality used in Series A, the subjects in Series B had to have a haemoglobin concentration of ≥12 g/100 ml blood, a plasma iron con

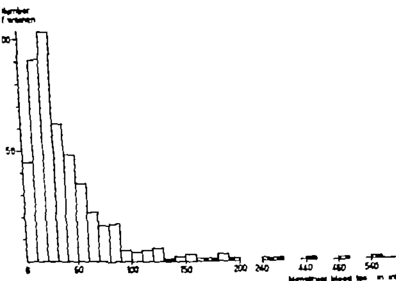


Fig. Distribution of menstrual blood loss in population sample from women living in Göteborg

centration of ≥ 80 μg/100 ml plasma, and a MCHC of ≥ 30 per cent. This series comprised 183 subjects. Fig. 3 shows the various subdivisions made to obtain Series A and B

The menstrual blood loss of Series A and Series B is shown in Table X. At all ages the mean values of both series were lower than in the original series (476 subjects) In Series A, the mean value of the menstrual blood loss was 38 5 ± 1 9 ml. Those who were excluded to obtain Series A, *i.e* women who considered themselves not healthy and/or not having a normal menstruation had a mean menstrual blood loss of 58.0 ± 7.0 ml (119 subjects) The difference between this value and that for Series A is statistically significant ($z = 2.677$ $p < 0.01$) Out of these 119 subjects, 82 were excluded only because they considered themselves not healthy for various reasons The mean menstrual blood loss in this group was 38 7 ± 4 1 ml. The difference between this value and the mean value of Series A was not statistically significant. 37 subjects were excluded because they did not consider their menstruation normal. Out of these 37 13 also did not con

Table X. *Mean Menstrual Blood Loss at Different Ages in Series A and B (for explanation see text)*

Age	5	3	3[illegible]	4[illegible]	45	5	Total
Series A							
Number of subjects	83	59	64	63	59	29	357
Mean value ± standard error of mean	34.8 ± 2 5	37.8 ± 4.3	36.5 ± 2.2	35.1 ± 2 9	39.0 ± 4 0	61 9 ± 15.7	38 5 ± 1 9
Median value	29 4	28.9	28 3	29.2	30 6	38 9	29.9
Difference between mean and median values	5.4	8 9	8 2	5 9	8.4	23 0	8 6
Series B							
Number of subjects	51	34	31	36	26	5	183
Mean value ± standard error of mean	32 9 ± 2 6	38 8 ± 4.8	29.7 ± 3 9	29.8 ± 3.0	31 7 ± 3 7	52.0 ± 7 5	33.2 ± 1 6
Median value	29 8	30 1	23 9	26.1	28 7	45 1	28 6
Difference between mean and median values	3 1	8 7	5.8	3.7	3.0	6 9	4 6

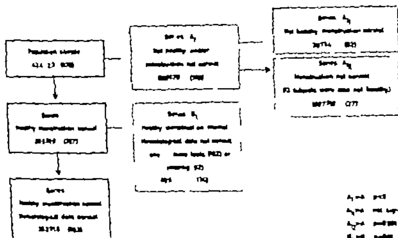


Fig. 3 Subdivisions of the series made to obtain Series A and B. The mean menstrual blood loss and the number of subjects are given for each series

sider themselves healthy The mean menstrual blood loss for the 37 women who considered their menstruation abnormal was 100 7 ± 19.4 ml. The difference between this latter value and the mean value for Series A was statistically significant ($z = 3.191$ $p < 0.001$)

Out of 439 women with subjectively normal menstruation 82 (18 7 per cent) did not consider themselves healthy compared with 13 (35.1 per cent) out of 37 with subjectively abnormal menstruation. The difference is statistically significant ($\chi = 5.78$ $p < 0.02$)

In Series B the mean menstrual blood loss was 33.2 ± 1 6 ml. The subjects excluded from Series A to obtain Series B had a mean value of 44.2 ± 3.5 ml. The difference is statistically significant (z 2.897 $p < 0.01$) The reasons for excluding 174 subjects from Series A to obtain Series B are given in Table XI.

Fig. 4 shows the distribution of the menstrual blood loss in Series A and B To facilitate comparison, the distribution histogram for the entire series is also graphed. The percentage of subjects with heavy blood loss was smaller in Series B than in Series A and smaller in Series A than in the original material.

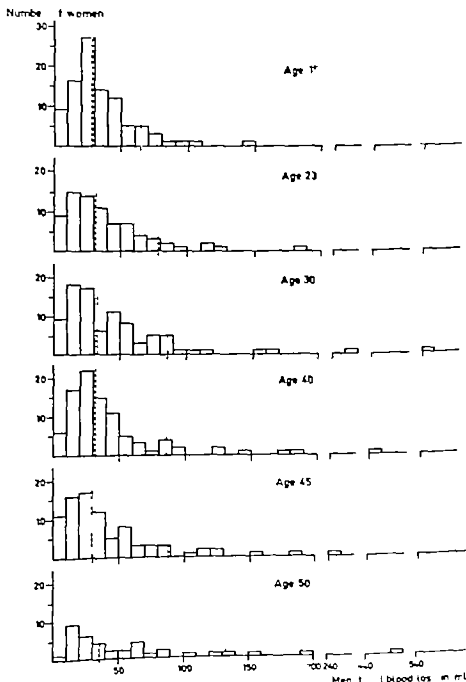


Fig. – Distribution of the menstrual blood loss in women at different ages (median and 90th percentile values indicated as dotted lines)

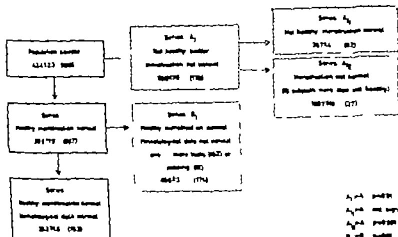


Fig 3 Subdivisions of the series made to obtain Series A and B The mean menstrual blood loss and the number of subjects are given for each series

sider themselves healthy The mean menstrual blood loss for the 37 women who considered their menstruation abnormal was 100.7 ± 19.4 ml. The difference between this latter value and the mean value for Series A was statistically significant ($z = 3.191$ $p < 0.001$)

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Table XI. *Reasons for Excluding Subjects from Series A to Obtain Series B*

Age	5	3	30	40	45	50	Total
Hæmoglobin below 12 g/100 ml blood	7	7	7	5	3	1	30
Plasma iron below 80 μg/100 ml plasma	11	5	12	6	6	7	47
MCHC below 30 per cent	3	1	2	1	7	2	16
Hæmoglobin below 12 g/100 ml blood and MCHC below 30 per cent	3	6	5	5	7	4	30
Hæmoglobin below 12 g/100 ml blood and plasma iron below 80 μg/100 ml plasma	2	4	–	4	5	7	24
Plasma iron below 80 μg/100 ml plasma and MCHC below 30 per cent	—	—	—	—	1	1	2
Hæmoglobin below 12 g/100 ml blood plasma iron below 80 μg/100 ml plasma and MCHC below 30 per cent	—	1	4	4	2	2	13
Values for hæmoglobin and/or plasma iron and/or MCHC are missing	6	1	1	2	2	—	12
Total	32	25	33	27	33	24	174

III *The menstrual blood loss in relation to hæmoglobin concentration plasma iron concentration and MCHC*

Besides measuring the menstrual blood loss the hæmoglobin concentration was determined in 474 subjects. The hæmoglobin concentration value was lacking in two subjects Their menstrual blood loss was calculated on the basis of 12 g/100 ml blood The plasma iron concentration was determined in 458 and MCHC in 465 subjects The reason why these values were not available for all the women was that there were technical failures in con nection with the determination lost blood samples or inability to obtain venous blood

The relations between the menstrual blood loss and the hæmoglobin concentration the plasma iron concentration, and MCHC are reported

a) as the mean value of these parameters in the following five ranges of blood loss 1–20 21–40 41–60 61–80 and $>$ 80 ml (see Fig 5)

b) as the number and percentage of subjects in each of these ranges having values below the normal limits chosen for the parameters *i e* a hæmoglobin concentration below 12 g/100 ml

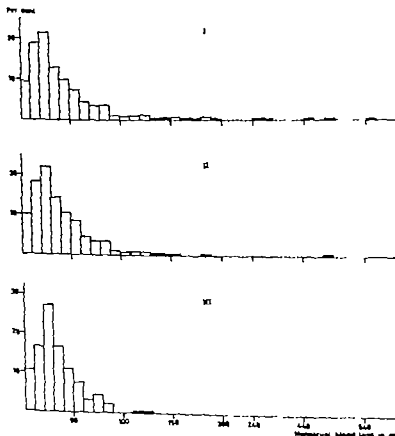


Fig. 4 Percentage distribution of the menstrual blood loss in I. the entire series II. Series A, and III. Series B (for explanation see text)

a plasma iron concentration below 80 μg/100 ml and a MCHC below 30 per cent (see Fig. 6)

Table XII shows the mean values of the haemoglobin concentration and the number and frequency of subjects with a haemoglobin concentration below 12 g/100 ml blood in the different ranges of menstrual blood loss. In the range 61–80 ml there was a decrease of the haemoglobin mean value. This decrease became more evident when the blood loss exceeded 80 ml. The difference between the mean values of this latter range and

Table XII *Hæmoglobin Concentration in Relation to Menstrual Blood Loss*

Range of Blood Loss in ml	Number of Subjects	Hæmoglobin Conc. (g/100 ml Blood) Mean Value ± Standard Error of Mean	Subjects with Hæmoglobin Conc. below g/100 ml Blood	
			Number	Per Cent
1—20	134	12.4±0.08	33	25
21—40	165	12.5±0.07	35	21
41—60	83	12.4±0.09	20	24
61—80	38	12.1±0.20	15	39
>80	54	11.4±0.17	36	67
Total	474	12.2±0.03	139	29

Table XIII. *MCHC in Relation to Menstrual Blood Loss*

Range of Blood Loss in ml	Number of Subjects	MCHC (Per Cent) Mean Value ± Standard Error of Mean	Subjects with MCHC below 30 Per Cent	
			Number	Per Cent
1—20	132	31.2±0.1	23	17
21—40	161	31.5±0.1	17	11
41—60	81	31.3±0.2	16	20
61—80	37	30.8±0.3	6	16
>80	54	30.8±0.3	19	35
Total	465	31.1±0.1	81	17

Indicates that the difference between this value and the value of the range 1—60 ml is statistically significant at the 5 per cent level.

Indicates that the difference between this value and the value of the range 1—60 ml is statistically significant at the 1 per cent level.

Indicates that the difference between this value and the value of the range 1—60 ml is statistically significant at the 0.1 per cent level.

the range 1–60 ml is statistically significant ($z=5.680$ $p<0.001$) The frequency of subjects with a hæmoglobin concentration below 12 g/100 ml blood was also higher in the ranges 61–80 ml and >80 ml. The difference between the frequency figures in these ranges and the range 1–60 ml is statistically significant ($z=2.189$ $p<0.05$ and $z=6.710$ $p<0.001$)

Table XIII shows the mean values of MCHC and the number and frequency of subjects with a MCHC below 30 per cent in the different ranges of blood loss. There was a significant decrease of the mean value of MCHC in the range 61–80 ml ($z=1.869$ $p<0.05$) which was more marked in the range >80 ... latter range the mean value of MCHC was also statis

Table XIV *Plasma Iron Concentration in Relation to Menstrual Blood Loss*

Range of Blood Loss in ml	Number of Subjects	Plasma Iron Conc. (µg/100 ml Plasma) Mean Value ± Standard Error of Mean	Subjects with Plasma Iron Conc. below 80 µg/100 ml Plasma	
			Number	Per cent
—20	28	08±3 3	21	16
—40	59	±2.7	39	25
4 —60	8	09±5 0	20	25
61—80	36	98±5 8	13	36
>80	54	87±5 7	24	44
Total	458	100± 8	1 7	26

indicates that the difference between this value and the value of the range 1—60 ml is statistically significant at the 5 per cent level

indicates that the difference between this value and the value of the range —60 ml is statistically significant at the per cent level

indicates that the difference between this value and the value of the range —60 ml is statistically significant t the per cent level.

tically lower than in the range 1–60 ml ($z=2.421$ $p<0.01$) The frequency of subjects with a MCHC below 30 per cent was statistically higher in subjects with a blood loss above 80 ml than in those loosing 1–60 ml ($z=3.615$ $p<0.001$)

Table XIV shows the mean values of the plasma iron con centration and the frequency of subjects with plasma iron con centration below 80 µg/100 ml plasma in different ranges of blood loss. The mean value was lower in the range 61–80 ml than in the range 1–60 ml, and still lower in the range >80 ml where the mean value was statistically lower than in the range 1–60 ml ($z=2.964$ $p<0.01$) With respect to the frequency of subjects with plasma iron less than 80 µg/100 ml plasma there was a significant increase in the range 61–80 ml ($z=1.898$ $p<0.05$) This increase was more marked in the group of females with a menstrual blood loss above 80 ml. In this latter range the frequency was also statistically higher than in the range 1–60 ml ($z=3.496$ $p<0.001$)

Fig. 5 shows the changes in the mean values of haemoglobin concentration, MCHC, and plasma iron concentration in the various ranges of menstrual blood loss. Fig. 6 shows the frequency of subjects below the selected normal limits of haemoglobin concentration, MCHC and plasma iron concentration in the various ranges.

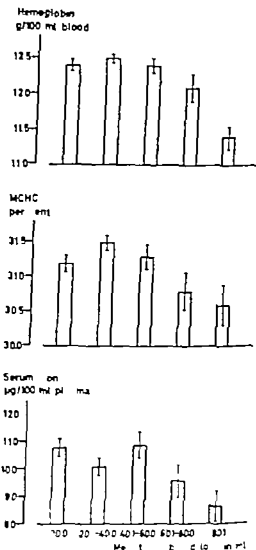


Fig. 5. Mean values of hæmoglobin concentration, MCHC and plasma iron concentration in different ranges of menstrual blood loss. The standard errors of means are indicated.

IV *Menstrual blood loss in relation to the subject s own judgment*

In the entire series 475 women stated whether they considered their menstrual blood loss scanty moderate or heavy To study the relation between the subjective judgement and the volume measured the series was divided into five groups according to the magnitude of blood loss The results are shown in Fig 7 The frequency of subjects, who considered their menstrual blood loss scanty decreased with an increasing blood loss while the reverse

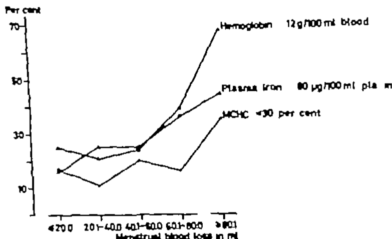


Fig. 6. Per cent of subjects with haemoglobin concentration below 1 g/ 00 ml blood, MCHC below 30 per cent, and plasma iron concentration below 80 μg/ 00 ml plasma in different ranges of menstrual blood loss

was found for those with a heavy blood loss. It is remarkable however that in the group with a blood loss exceeding 80 ml, 37 per cent (19 subjects) considered their menstrual blood loss moderate and 4 per cent (2 subjects) considered it even scanty In the group with a menstrual blood loss of less than 20 ml, however 14 per cent (19 subjects) considered their menstrual blood loss heavy

DISCUSSION

The individual constancy of the menstrual blood loss (for references see Hallberg and Nilsson, 1964 b) makes it important to get more exact information on the variation of the menstrual blood loss in a population. Such information is essential in clinical practice and when evaluating the iron balance in women. Knowledge of the variation of the menstrual blood loss in a population can hardly be gained from previous studies, in which there were special selections of subjects for investigation Moreover it is difficult to make a comparison with previous studies due to the heterogenity with respect to methods age distribution,

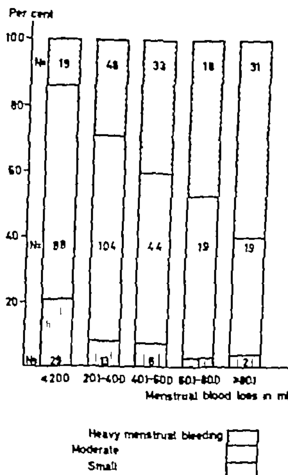


Fig 7 Subjective judgments of menstrual blood loss.

etc. The present study comprises measurement of the menstrual blood loss in a large series selected at random from a population.

The main difficulty in studying such a large series is to ensure that each subject follows the instructions given for collecting the menstrual blood. In the present investigation an approximate evaluation of how the instructions were followed was obtained by registering the consumption of tampons and towels. The extraneous waste of blood was probably reduced if tampons and towels were used simultaneously as the mean blood loss was lower for those using only towels than for those using both towels and tampons. The difference however is not statistically significant.

The sampling technique used in the present study has the advantage that differences between the magnitude of menstrual blood loss at different ages may be discovered more easily. A disadvantage with the sampling technique used is that the results may not be representative for the population as a whole. However, the difference in menstrual blood loss between adjacent age-groups studied was small. Thus, it is reasonable to assume that there will not be pronounced deviations at ages between those studied. The material used may thus be considered fairly representative for the whole population.

Out of the women selected who were still living in the city at the time of the examination, 54 (7.2 per cent) did not participate. This frequency of non-response must be considered low for studies of this kind. The most frequent reason for not participating was lack of time due to employment, custody of children, etc. Judging from repeated interviews the reasons given seemed to be valid. Nothing indicated that the magnitude of the menstrual blood loss had any influence on the inability or refusal to participate. Hence, systematic errors were probably not introduced by the non-response.

The menstrual blood loss was not measured in 35 subjects (6.8 per cent of the menstruating women examined). Out of these 35, 14 refused due to reasons of assumed discomfort and 12 were not available when the containers were to be collected. Judging from the menstrual history and the detailed reasons given by the non-participants, it does not seem probable that the menstruation in these women differed from the rest of the population.

A comparison between various ages shows that the mean and median values of the menstrual blood loss of women aged 50 years are higher than at other ages. This is probably due to the higher frequency of pathological conditions, such as fibroids of the uterus and metropathia haemorrhagica cystica, which are more common during the pre-menopausal years.

The mean and median values of the menstrual blood loss for subjects aged 15 years are lower than for the other age-groups. However, the values for the 15-year-old girls were influenced by the fact that many of them did not use tampons for the collection of the menstrual blood. For this reason the waste of blood

might be greater in the 15 year group than in the others. The difference between the mean value for those in the 15 year group using tampons and towels and the corresponding value at the ages 23–45 years is not statistically significant. Thus the low mean value in the 15 year group in the population sample is probably mainly due to an inadequate collection technique It cannot be excluded, however that other factors such as differences in e.g. parity and sexual activity also may be of importance Further studies are needed to clarify these aspects

NORMAL LIMITS FOR THE MENSTRUAL BLOOD LOSS

The difficulty of defining any biological normal limits also exists in the case of the menstrual blood loss Normal values are often defined as the values observed in healthy subjects However the values for menstrual blood loss obtained in healthy subjects are not necessarily normal because health may be defined in various ways and because a loss exceeding a certain value may later on lead to a non healthy state

Methodological aspects

Using statistical methods the limits for the normal menstrual blood loss were studied in the entire population sample and in groups of subjects fulfilling various criteria of "health As the main consequence of heavy menstrual blood loss is a development of iron deficiency attempts were made to establish the upper normal limits by studying the relationship between the magnitude of the menstrual blood loss and various parameters reflecting the iron state of the body

One series studied (Series A) comprised all subjects who considered themselves healthy and who considered their menstruation normal The other series (Series B) included only those subjects in Series A who also had a hæmoglobin concentration of ≥ 12 g/100 ml blood plasma iron ≥ 80 μg/100 ml plasma and a MCHC ≥ 30 per cent.

These limits were used to exclude most subjects with iron deficiency Of course it is impossible to set a certain limit above

which all are normal and below which all have iron deficiency Most workers consider a haemoglobin concentration of 12 g/100 ml blood the lowest normal value in fertile women (World Health Organization Technical Report Series 1959) A MCHC of 30 per cent and a plasma iron concentration of 80 μg/100 ml were chosen as reasonable limits between a normal and an iron deficient state (Wintrobe, 1961) These limits are of course not free from objections but they imply a reasonable separation between groups having significantly different frequencies of iron deficient subjects. The limit value 30 per cent for MCHC may be considered low but the haematocrit values were not corrected for trapped" plasma.

The wide normal ranges of the haemoglobin concentration, the plasma iron concentration and the MCHC involve a risk that even some normal subjects were excluded and that subjects with a slight degree of iron deficiency were included. However an exclusion of some normal subjects will probably not appreciably affect the mean values of the series.

The effect of using various criteria of normality on the menstrual blood loss distribution curve is shown in Fig. 4 In Series A, the main effect on the skewness of the distribution was obtained by excluding subjects considering their menstruation abnormal The mean value in this excluded group was as high as 100 6 ml.

The mean value for those excluded only because they did not consider themselves healthy was 38 7 ml, which might imply that the state of subjective health as such was unrelated to the magnitude of the menstrual blood loss. However the frequency of subjectively non-healthy subjects was statistically higher in the group having abnormal menstruation (35.1 per cent) than in the group having normal" menstruation (18 7 per cent) This finding indicates that abnormal menstruation which for most subjects was equivalent to heavy menstrual blood loss is often associated with a subjectively non-healthy state This observation necessitates further studies It may be concluded that both criteria used for Series A i.e. normal menstruation and subjective health, are valid.

To get Series B from Series A 174 subjects who had one or more haematological values below certain limits were excluded.

This reduced the mean menstrual blood loss from 38.5 (Series A) to 33.2 ml (Series B) The average loss in these 174 subjects was 46.3 ml However it is important to point out that to obtain Series A from the entire series 59 subjects with hæmatological values below the limits chosen were already excluded because they considered themselves not healthy and/or considered their menstruation abnormal

Upper normal limit

The normal limits are usually defined as the mean value ± two standard deviations However this definition is valid only if the values are normally distributed. Due to the obvious skewness of the distributions in the present study it was impossible to use this method.

The skewness can at least partly be explained by an increased frequency of values from abnormal subjects in the right tail of the distribution curve as the skewness was reduced in Series A and B (see Fig 3) The skewness of the entire population sample and its reduction in Series A and B may be illustrated by the successively decreasing difference between the mean and median values (Table VIII and X)—13.4 8.6 and 4.6 ml. Due to the skewness still present in Series B an estimate of the upper normal limit is obtained by calculating the 95th percentile value of Series B This value, 76.4 ml is probably a fairly good estimate of the upper normal limit of the menstrual blood loss as it is consistent with the finding of an increased frequency of iron deficiency in subjects with menstrual blood loss in the ranges 61–80 ml and above 80 ml

The definition of normality of the menstrual blood loss in the present study was based mainly on the effect of the blood loss on the iron balance Because of that the upper normal limit will be dependent on the average intake of iron by the population. The value obtained is thus not applicable to every population. Detailed dietary histories were taken in most of the subjects. The average daily intake of iron in women 15–50 years was 10.2 mg This study will be reported elsewhere.

In the present study no consideration was taken of the in

dividual dietary intake of iron or of whether or not extra iron was supplied. Nor were other individual factors considered which could affect the iron balance, e g frequency of pregnancies and menstrual intervals.

Comparison between the present and previous studies

Compared with the findings of other investigators the mean value of the menstrual blood loss of the entire series expressed as ml blood is lower than the mean value found by Barer and Fowler (1936) Arens (1945) and Göltner and Galler (1964) but higher than the mean value found by Leverton and Roberts (1937) Baldwin, Whalley and Pritchard (1961) Hagedorn, Kiely Tauxe, and Owen (1962) Apte and Venkatachalam (1963) Hytten, Cheyne and Klopper (1964) and Jacobs and Butler (1965) The differences may be due partly to the fact that the various studies are different with respect to the selection and the number of subjects studied, their age, parity etc. Furthermore the methods of collecting blood and estimating the menstrual blood loss were different. Another factor which may have affected the mean values in the previous studies is the inclusion in some series of subjects with hemoglobin values below normal limits (Barer and Fowler 1936 Arens 1945) The importance of excluding anaemic subjects is evident from the present study and from the results obtained by Hagedorn, Kiely Tauxe, and Owen (1961) and Jacobs and Butler (1965)

With the same methods as used in the present study Hallberg and Nilsson (1964 b) found a mean value of 28 ml in a series of 12 healthy nursing students (average age 22 years) studied during 12 consecutive periods. This value is lower than the mean value of the 23-year-group in Series B of the present study It can be assumed that the collection of towels and tampons was done quite rigourously by the nursing students, who were aware of the importance of avoiding any waste of blood. The fact that the mean value of the present study is higher than for the 12 nursing students may indicate that the collection in the present study was adequate Hallberg, Nilsson, Hög

dahl and Rybo (1966) found a mean value of 34 ml in female industry workers a value which is in good agreement with the value of 33 2 ml obtained in the present study for non anæmic subjects who considered themselves healthy and their menstruation normal (Series B)

The skewness of the distribution in all ages in the present study is in accordance with the results of other authors (Barer and Fowler 1936 Millis 1951 Baldwin, Whalley and Pritchard, 1961 Hytten, Cheyne, and Klopper 1964)

The findings in the present study that an increased number of subjects with signs of iron deficiency occurred in the ranges above 60 ml and that in these ranges the average values of hæmoglobin and plasma iron concentration and MCHC were lower than in the ranges below 60 ml are in principle in accordance with the results of Arens (1945) who reported a negative correlation between the hæmoglobin concentration and the menstrual blood loss and with Hagedorn Kiely Tauxe and Owen (1961) and Jacobs and Butler (1965) who found a larger blood loss in iron deficient subjects than in normal women. Other investigators (Barer and Fowler 1936 Millis 1951 Baldwin Whalley and Pritchard 1961 Rankin, Veall Huntsman and Liddell 1962) could not show any relationship between the hæmoglobin concentration or hæmatocrit and the magnitude of menstrual blood loss

Subjective judgement of menstrual blood loss

The difficulties of evaluating the menstrual blood loss on the basis of the patient s own judgment are well known. The results obtained in the present study emphasize this fact. Thus 40 per cent of women with a blood loss above 80 ml considered their menstruation moderate or even small. The number of women who considered their menstruation heavy on the other hand, increased with increasing blood loss and the mean value for those who considered their menstruation abnormal is considerably higher than for the others Further investigations are being made to analyse the accuracy of anamnestic data.

CONCLUDING REMARKS

The results of the present study indicate that the upper normal limit of the menstrual blood loss is situated in the range 60–80 ml. This estimation can be compared with an evaluation of the tolerable menstrual blood loss calculated from available iron balance data. The Food and Nutrition Board (1963) recommended a daily intake of 15 mg of iron for women in the fertile ages. Assuming a daily intake of iron of this magnitude and an absorption of 10 per cent (Moore, 1955) the daily amount of iron absorbed will be 1 5 mg. Finch (1959) found that the daily loss of iron in non-menstruating women was about 0 6 mg daily Thus 0.9 mg iron is left to cover the menstrual iron losses. With a haemoglobin concentration of 12 g/100 ml blood and an interval of 28 days, the iron balance will be maintained with menstrual blood losses up to 63 ml. This rough estimation is thus in agreement with the upper normal limit calculated on the basis of the data in the present study However these calculations stress the fact that the upper limit of the normal menstrual blood loss can not be given exactly as the dietary intake of iron must also be taken into consideration. However the results obtained in the present study show the magnitude of menstrual blood loss which can be tolerated with a maintained iron balance

SUMMARY

The menstrual blood loss and its variations were studied in a series of 476 females in Göteborg aged 15, 23, 30 40 45, and 50 years and selected at random.

7 5 per cent of the selected women failed to cooperate in the investigation. Out of the females examined 22.9 per cent had amenorrhoea. Measurement of the menstrual blood loss was made in 93.3 per cent of the menstruating women.

The mean value of the menstrual blood loss was 43.4 ± 2.3 ml in the entire series. There were no great differences in amount of blood lost between different ages except in the 15 year-group which had the smallest and in the 50-year group which had the highest mean value of menstrual blood loss.

In healthy subjects considering their menstruation normal the

mean value was 38 5 ± 1 9 If subjects with signs of iron de ficiency were excluded the mean value was reduced to 33.2 ± 1 6 ml

On the basis of statistical analyses of the different series and a study of the frequency of signs of iron deficiency in different ranges of menstrual blood loss it was concluded that the upper normal limit of the menstrual blood loss is situated between 60–80 ml and that a blood loss above 80 ml should be regarded as pathological

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mean value was 38.5 ± 1.9. If subjects with signs of iron deficiency were excluded the mean value was reduced to 33.2 ± 1.6 ml.

On the basis of statistical analyses of the different series and a study of the frequency of signs of iron deficiency in different ranges of menstrual blood loss it was concluded that the upper normal limit of the menstrual blood loss is situated between 60–80 ml and that a blood loss above 80 ml should be regarded as pathological.

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Med. dr. Tage Larsson has given valuable advice with respect to the sampling method, for which we wish to express our sincere gratitude. The statistical treatment was reviewed by fil. mag. Håkan Lindstrom and fil. kand. Lennart Gustavsson.

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URINARY EXCRETION OF HORMONES DURING THE CLIMACTERIC

BY

MIRJAM FURUHJELM

Ageing of the ovaries starts early in life. There is a progressive decrease in the number of follicles starting before the menarche and continuing during the fertile period. The climacteric is brought on by a progressive decline in ovarian function, depending on the decreasing number of follicles and of a decrease in the vascular supply. The hormonal function of the ovaries after the cessation of the menses is still open to question, but the stroma of the ovaries is capable of producing hormones even in the absence of follicles and corpora lutea, according to Ryan and Smith (1965). After the menopause there is still an excretion of œstrogens in the urine but whether this is of ovarian or adrenal origin is not known.

Novak (1947) not infrequently finds a typical hyperplasia of the endometrium identical with the one seen during reproductive age in women far beyond the menopause. He supposes that the source of this postmenopausal œstrogen is extraovarian, probably the adrenal cortex. Hertig (1944) has investigated ovaries taken from women of postmenopausal age. He suggests that the stroma cells in the ovaries are able to metabolise œstrogens. They are stimulated by the elevated secretion of gonadotrophins from the pituitary. Lajos *et al.* (1963) found histologic changes in the ovaries compatible with œstrogen production in women with hyperœstrogenism after the menopause. Histological examination showed in every case diffuse or nodular theca cell hyperplasia. An increase in serum lipid levels and a

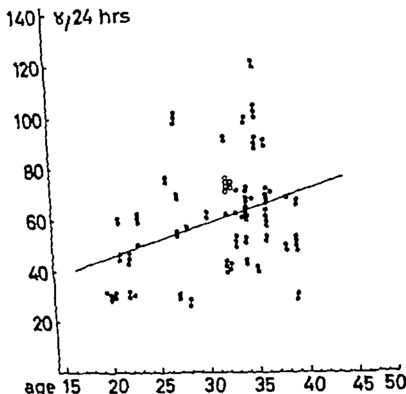


Fig. *Menstruating women*. Excretion of oestrogens. Positive correlation between age and excretion of oestrogens P< oo y=40.2 +0.64

greater incidence of coronary heart disease after the menopause have been documented in several studies. Anatomic studies have shown less coronary atherosclerosis in women with intact ovaries than in castrated women. Premature castration results in an increased prevalence of clinical atherosclerosis and coronary heart disease (Robinson *et al.* 1959, Oliver and Boyd 1959 Randall *et al.* 1964)

For the clinicians it is of great importance to know whether or not the ovaries are responsible for production of oestrogens after the menopause The output of oestrogens in the urine after the menopause has been examined by Jaller (1948) who

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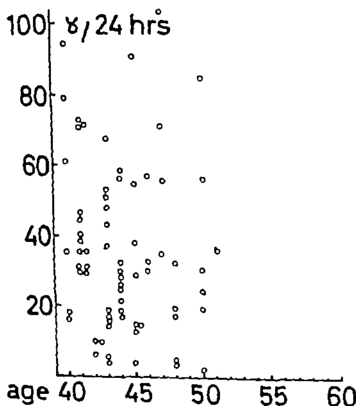


Fig. 3. Pre-menopausal women. Excretion of oestrogens. Excretion of gonadotrophins normal. Excretion of pregnanediol 2.5 mg/24 hrs. No correlation between age and excretion of oestrogens.

during the climacteric and after the menopause we have determined the urinary excretion of gonadotrophins, oestrogens, pregnanediol and 17-ketosteroids in the urine in a series of premenopausal and postmenopausal women. For our investigation we have divided the pre- and postmenopausal period into three stages.

1. The function of the corpus luteum decreases. There is a decrease of the postovulatory excretion of the pregnanediol to the values normally found in the preovulatory phase of the menstrual cycle. The women still have regular periods, but these

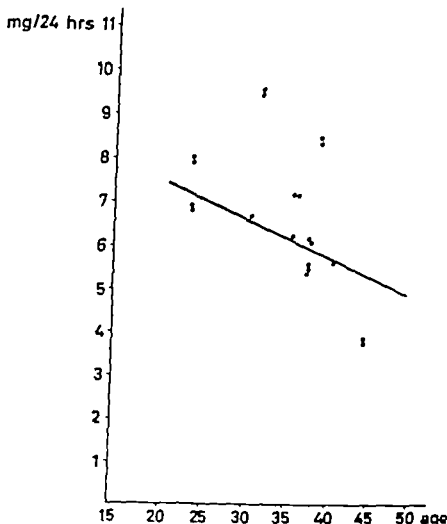


Fig. 2. *Menstruating women.* Excretion of 17-ketosteroids. Negative correlation between age and excretion of 17-ketosteroids. $P < 0.001$ $y = 7.97 - 0.06$

found a 24 hrs excretion of 10–26 μg for those who had passed the menopause a year previously. Four years after the menopause the excretion was 5–6 μg per 24 hrs, and after 7 years he found insignificant values. Using Brown's method McBride (1957) found, that measurable amounts of oestrogens, 2–20 μg, are excreted by women after the cessation of menses at the menopause. The amounts excreted varied greatly in different individuals and even from day to day in the same person.

In order to evaluate ovarian function a few years before and

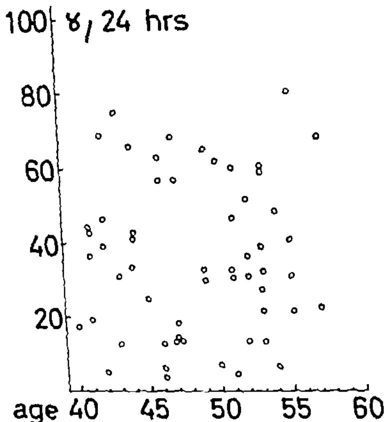


Fig. 5. Pre-menopausal women. Excretion of oestrogens. Excretion of gonadotrophins elevated. Excretion of pregnanediol < 5 mg/24 hrs. No correlation between age and excretion of oestrogens.

menstruating women with a normally functioning corpus luteum and 3 groups of pre- and postmenstrual women divided into the stages mentioned above

1. Fifty three normally menstruating women 15–50 years of age 198 determinations were made in the ovulatory and secretory phases of the menstrual cycle

2. Seventy-two women aged 40–53 years who still had periods which sometimes were shortened. 75 determinations. The excre-

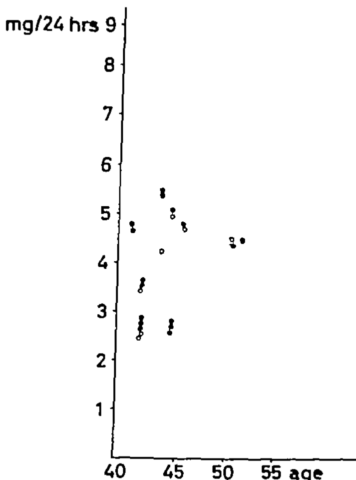


Fig 4 *Pre-menopausal women* Excretion of 17-ketosteroids Excretion of gonadotrophins normal Excretion of pregnanediol 2 5 mg/24 hrs No correlation between age and excretion of 17 ketosteroids

are sometimes shortened. The excretion of gonadotrophins in the urine is within normal limits

2. The periods appear less frequently and often irregularly The excretion of gonadotrophins in the urine is elevated.

3 There is a cessation of menstruation The excretion of gonadotrophins in the urine is elevated.

Material

Our material consists of 273 healthy women, aged 20–78 years. They were divided into 4 groups *i e* one group of normally

received no oestrogen therapy for the last 6 months or longer 98 determinations. The excretion of gonadotrophins was elevated.

Methods used

Gonadotrophins (Hamburger 1933)
Œstrogens (Furuhjelm and Waller 1958)
Pregnanediol (Klopper *et al.* 1955)
17-ketosteroids (Vestergaard, 1951)

The urine was collected in group 1 from healthy volunteers, in groups 2 3 and 4 from women attending the out-patients department for a health control, and also from otherwise healthy women who were in hospital because of prolapse or stress incontinence In all cases we determined the excretion of gonadotrophins, oestrogens pregnanediol and 17-ketosteroids in the same sample of urine (collected during 24 hrs)

Results

Group 1

a) oestrogens. (fig. 1) Mean excretion of oestrogens 61 5 ± 1 7 µg/24 hrs. There is a statistically significant increase of the oestrogenic excretion with increasing age ($P < 0.001$) Regression line $y = 40\ 21 + 0\ 64$

b) *17 ketosteroids* (fig. 2) Mean excretion of 17-ketosteroids 6 15 ± 2.8 mg/24 hrs. There is a statistically significant decrease in the excretion of 17-ketosteroids with increasing age ($P < 0\ 001$) y 7.97 − 0 06

Group 2

a) *oestrogens.* (fig 3) The excretion of oestrogens has a mean value of 35.2 ± 6 µg/24 hrs. The excretion of oestrogens has no correlation with the age of the women.

b) *17-ketosteroids.* (fig. 4) The mean excretion of 17 ketosteroids is 4.33 ± 2.2 mg/24 hrs. The excretion of 17-ketosteroids is not correlated with the age of the women.

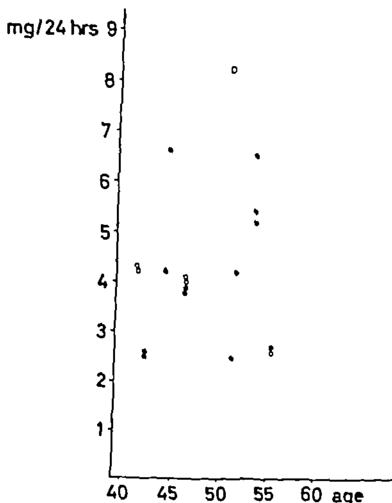


Fig 6. *Pre-menopausal women.* Excretion of 17 ketosteroids. Excretion of gonadotrophins elevated. Excretion of pregnanediol 2.5 mg/24 hrs No correlation between age and excretion of 17 ketosteroids.

tion of gonadotrophins was not elevated.

3 Fifty-one women aged 40–53 years who still had periods, but less frequently and sometimes irregularly 57 determinations were made The excretion of pregnanediol was less than 2.5 mg/24 hrs on the 22nd day of the cycle The excretion of gonadotrophins in the urine was elevated.

4. Ninety-seven women aged 45–78 years who had not menstruated for more than a year or longer and who have

received no oestrogen therapy for the last 6 months or longer 98 determinations. The excretion of gonadotrophins was elevated.

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Results

Group 1

a) *oestrogens.* (fig. 1) Mean excretion of oestrogens 61.5 ± 1.7 μg/24 hrs. There is a statistically significant increase of the oestrogenic excretion with increasing age ($P < 0.001$). Regression line $y = 40.21 + 0.64$

b) *17-ketosteroids.* (fig. 2) Mean excretion of 17-ketosteroids 6.15 ± 2.8 mg/24 hrs. There is a statistically significant decrease in the excretion of 17-ketosteroids with increasing age. ($P < 0.001$) $y = 7.97 - 0.06$

Group 2

a) *oestrogens* (fig. 3) The excretion of oestrogens has a mean value of 35.2 ± 2.6 μg/24 hrs. The excretion of oestrogens has no correlation with the age of the women.

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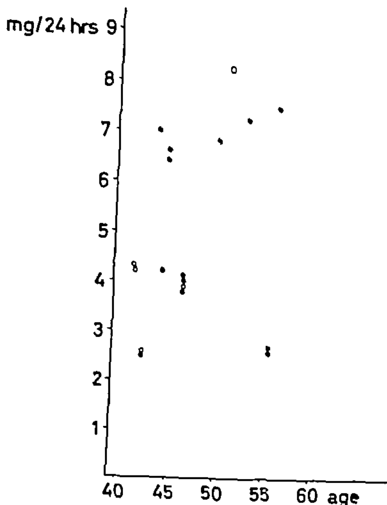


Fig 6. *Pre-menopausal women* Excretion of 17 ketosteroids. Excretion of gonadotrophins elevated. Excretion of pregnanediol ≥ 5 mg/24 hrs No correlation between age and excretion of 7 ketosteroids

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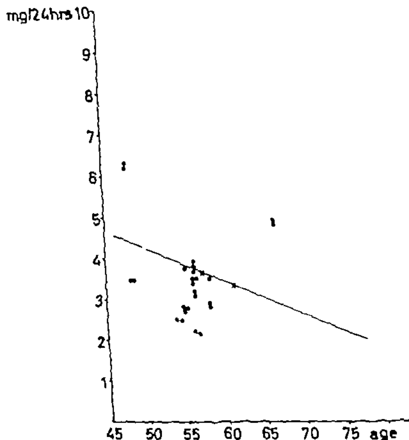


Fig. 8. Menopausal women. Excretion of 17-ketosteroids. Negative correlation between age and excretion of 17-ketosteroids. $P < 0.00$ $y = 8.19 - 0.08$

b) 17-ketosteroids (fig. 8). The excretion of 17-ketosteroids decreases with increasing age. The decrease is statistically significant ($P < 0.001$) $y = 8.19 - 0.08$. The mean value of the excretion of 17-ketosteroids is 3.69 ± 2.0 mg/24 hrs.

Discussion

It is notable that the excretion of oestrogens rises with increasing age when menstruation is still normal. Brown (1958) has

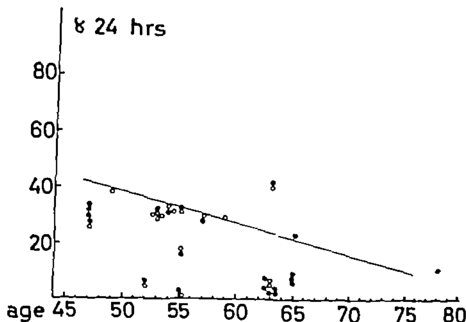


Fig 7 *Menopausal women.* Excretion of œstrogens. Negative correlation between age and excretion of œstrogens $P<0.01$ $y=73.31-0.78$

Group 3

a) *œstrogens* (fig 5) The mean excretion of œstrogens is 36.1 ± 2.2 μg/24 hrs and has no correlation with the age of the patient.

b) *17 ketosteroids* (fig 6) The mean value of the excretion of 17-ketosteroids is 4.3 ± 2.4 mg/24 hrs The excretion has no correlation with the age of the women.

Group 4

a) *œstrogens* The excretion of œstrogens in the urine decreases with increasing age (fig 7) There is a significant correlation between the age of the women and the amount of œstrogen excreted. ($P<0.01$) $y=73.31-0.8$ The mean excretion of œstrogens is 28.1 ± 2.0 μg/24 hrs This figure is lower than in group 1 the difference being highly significant ($P<0.001$) The mean excretion of œstrogens is also lower than in groups 2 and 3, the difference being probably significant ($P<0.05$) Also there are some values which correspond to those found in normally menstruating women.

difference in the excretion of oestrogens in castrated women and in postmenopausal women. At present such a study is being carried out using gas chromatography.

In any case there is an excretion of oestrogens postmenopausally until the age of 65–70 years. This excretion may explain the decreased rate of vascular diseases in elderly women in comparison with men of a corresponding age. A prophylactic oophorectomy in order to prevent a future carcinoma of the ovary may therefore increase the risk of death from vascular disease and thus shorten her life instead of lengthen it. To what extent an artificial substitution of oestrogens can counteract this outcome we do not know.

SUMMARY

The excretion of gonadotrophins, oestrogens, pregnanediol and 17-ketosteroids in the urine has been determined in 273 healthy women, 20–78 years old. They were divided into 4 groups. *Group 1* included 53 normally menstruating women with normal corpus luteum function. *Group 2* consisted of 72 women who still had periods, but a failure of luteal function. The excretion of gonadotrophins in the urine was normal. *Group 3* included 51 women who also still had periods and a failure in the luteal function but where the excretion of gonadotrophins in the urine was elevated. *Group 4* included 97 postmenopausal women.

In *group 1* the excretion of oestrogens in the urine increased with increasing age. The excretion of 17-ketosteroids on the contrary decreased with increasing age. In *groups 2 and 3* the excretion of oestrogens was not correlated with the age and was lower than in the group of normally menstruating women. The excretion of 17-ketosteroids had no correlation with the age of the women. In *group 4* the excretion of oestrogens in the urine decreased with increasing age. The mean excretion of oestrogens was lower than in groups 1, 2 and 3, but some values of oestrogen were found which corresponded to amounts found among normally menstruating women. The excretion of 17-ketosteroids decreased with increasing age.

found notable variations in the excretion of œstrogens from one woman to another but he has not correlated them according to age Speculating on the reason of this increase with age one can suggest that with increasing age more œstrogens are needed in order to bring about an adequate release of gonadotrophins to produce ovulation When the function of the corpus luteum decreases, much smaller amounts of œstrogens are excreted. The excretion of œstrogens in groups 2 and 3 where the function of the corpus luteum has ceased is lower than in the group of normally menstruating women the difference being highly significant.

On the contrary the excretion of 17-ketosteroids decreases with increasing age, and is not related to the ageing of the ovaries

No difference is found between the excretion of œstrogens in group 2 where the excretion of gonadotrophins is not elevated, and in group 3 where the women have an elevated excretion of gonadotrophins. As long as the women have enough œstrogens to produce adequate changes in the endometrium and in the blood vessels menstruation continues irrespective of whether or not the excretion of gonadotrophins is elevated.

Postmenopausally the excretion of œstrogens decreases progressively and is probably nil at the age of 70. However Brown's method usually gives falsely high values when only small amounts of œstrogens are present in the urine This depends upon the difficulty of removing the chromogens from the urine Post menopausally sometimes the excretion of œstrogens may reach almost the same value as in normally menstruating women. Probably these women represent cases of hyperœstrogenism which Lajos *et al* (1963) have described.

The excretion of 17-ketosteroids decreases with increasing age. It seems to be independent of ovarian function. It is highest at the age of 20 years and decreases thereafter progressively

The conclusion that the œstrogens in the postmenopausal age originate from the ovaries is not justified solely on the results of this investigation. Neither is it possible to estimate small amounts of œstrogen with the method used. A more sensitive method probably can answer the question whether there is any

difference in the excretion of oestrogens in castrated women and in postmenopausal women. At present such a study is being carried out using gas chromatography

In any case there is an excretion of oestrogens postmenopausally until the age of 65–70 years. This excretion may explain the decreased rate of vascular diseases in elderly women in comparison with men of a corresponding age. A prophylactic oophorectomy in order to prevent a future carcinoma of the ovary may therefore increase the risk of death from vascular disease and thus shorten her life instead of lengthen it. To what extent an artificial substitution of oestrogens can counteract this outcome we do not know

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FUNCTION OF THE URINARY BLADDER AFTER SUBTOTAL HYPOGASTRIC SYMPATHECTOMY

BY

AUNE SALLINEN

Presacral denervation is often used as an adjunct to other therapeutic methods in various gynaecologic painful conditions and sometimes in chronic irritability of the urinary bladder In Finland the best known technique is the method evolved by Cotte 1949. However as the results indicate room for improvement, many workers (e g Held, 1943 Thiermann 1948 Vara, 1950) now employ various extensions of this operative method. For instance in addition to hypogastric sympathectomy the sympathetic trunk is excised either bilaterally or only on the right side between ganglia L--S Alternatively these ganglia are removed bilaterally as well as the sympathetic trunk between them (Vara 1950) Some surgeons extend the operation caudally and in addition to the hypogastric ganglia resect also the nervi erigentes (= nervi pelvici) which bring parasympathetic fibres from sacral roots II IV into the urinary bladder (Thiermann 1948)

While denervation operations produce either relief or an improvement in the painful condition in some of the patients according to the literature they are sometimes followed by disturbances in the function of the urinary bladder It is therefore important to have accurate knowledge of the innervation of the bladder before undertaking these operations. This subject will not be dealt with in the present article A detailed description can be found in many text books and in publications by e g Dennig, 1929 and Vara 1950

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Received on Febr 2nd 1966

Ovarian retention cyst	1	patients
Parovarian cyst	1	
Uterine myoma	3	"
Uterine hypoplasia	1	
Amenorrhoea	1	
Parametritis posterior	1	
Fixed retroversion	1	
Pelvic neuralgia	1	"
Condition after toxemia	1	"

The operations in connection with which subtotal hypogastric sympathectomy was performed were

Ventrisuspension (Grossen-Gilliam method)	11	patients
(Bonney Berkeley method)	1	"
(Baldy Webster method)	1	"
(Menge method)	1	"
Insertion of Polyethylene rods	11	
Cervical dilatation	3	
Oophorectomy	8	
Salpingo-oophorectomy	6	
Salpingostomy	5	
Salpingolysis	4	
Salpingectomy	2	
Freeing of adnexal adhesions	7	"
Implantation of tubes in uterine cavity	4	"
Implantation of ovaries in uterine cavity	2	"
Ovarian resection	4	"
Tubal	1	"
Removal of parovarian cyst	1	"
Puncture of ovarian cysts	2	"
Myomectomy	3	
Excision of endometriotic foci	4	"
Resection of uterosacral ligament	4	"
Division of intraperitoneal adhesions	3	
Appendicectomy	7	"
Inversion of appendix	4	"

The sympathetic innervation of the urinary bladder still consti tutes the great unknown quantity in the physiology of micturition If the sympathetic nerves are excised, as in Cotte s opera tion according to Langreder 1961 the condition of the blad der does not change while Dennig 1929 reports that the detrusor tone is raised and the capacity of the bladder de- creases as the parasympathetic effect is inhibited. On the other hand if the nervi erigentes (= nervi pelvici) are excised in addi *tion to the hypogastric nerves as performed by Thiermann* 1948 there should ensue in Dennig s 1929 opinion atony of the bladder and later reflex micturition. But if the nervi pelvici are removed only unilaterally in connection with the resection of the *hypogastric sympathetic nerves no notable disturbances ensue in* the urinary bladder Disturbances in sensitivity have sometimes been encountered in the urinary bladder after extensive presacral hypogastric nerve resections (Kaser and Iklé 1964) None of the 65 patients of Varas 1950 series showed disturbances of urinary bladder function.

Material

Professor Vara made available to me 30 of his private patients on whom he performed Cotte Vara sympathectomy chiefly in connection with reparative surgery for various causes of primary (26) or secondary (4) sterility in 1959–1964

The patients mean age was 30 1/2 years.

The causes leading to sterility were as follows

Endometriosis	5	patients
Intramural tubal occlusion	4	
Peritubal occlusion	1	"
Spasmodic dysmenorrhoea	2	
Adnexal adhesions	3	
Chronic salpingitis	2	
Tuberculous salpingitis	1	
Tubal mole	1	
Polycystic ovaries	[illegible]	
Ovarian adenofibroma	1	

each aliquot, the tube leading from the bottle was closed and the height of the column of fluid in the glass tube was recorded when oscillation was minimal. This bladder pressure adaption value was considerably lower than the first value although the variations caused by respiration (1–2 cm) were the same

The adaption values are considered to be the most reliable in dicators of bladder function but the instillation speed should not be to high. However to obtain an accurate idea of the shape of the cystometrogram, the cystometric determinations should be be more frequent than after every 50 c. c. (Simeone and Lampson 1937) Aliquots of 50 c. c. have generally been used, how ever and for practical reasons were employed in the present work as well. The time within which the urinary bladder adapts to the different values was not considered. During the measurement, the patient was asked to state when she felt 1) filling of the bladder 2) urinary urgency and 3) intensive unpleasant urinary urgency and 4) the moment when she found it difficult to retain urine She was then asked to strain and the micturition pressure was measured. The micturition pressure recorded was thus the com bination of the pressure produced by the detrusor muscle and the intra-abdominal pressure exerted by the abdominal muscles.

The micturition pressure exerted by the detrusor muscle alone cannot be obtained until the intra abdominal pressure is measured concurrently from the rectum during cystometry and the reading obtained is deducted from the total bladder pressure (Lattmer *et al.* 1964) Although only cystometry war per formed on the patients the results are comparable as the same procedure was followed for all the patients. Because the denervation performed does not affect the function of the abdominal muscles the postoperative changes in micturition pressure are due mainly to changes in the activity of the detrusor muscle The tenderness of the operation wound may have an inhibitory effect on active elevation of intra-abdominal pressure by the abdominal muscles in the first postoperative cystometry

Method of Investigation

The principal aim of cystometry is the study of the function of the detrusor muscle Therefore it is a suitable method for following post-denervation bladder pressure and was used in the patients of the present series.

The first cystometric measurement was made on the evening before operation the second an average of 8 days after the opera tion and for 10 patients a third measurement was made an average of 1 year 10 months postoperatively The shortest inter val was 1 month 4 days and the longest 4 years 3 1/2 months

A water manometer of the type used by most workers since 1934 (Watkins 1934) and by Dubois ever since 1876 was employed for cystometry With the increase in knowledge of the physiology of the bladder the apparatuses for studying its function have improved and even mild functional disturbances of the urinary bladder can now be detected. The newest instruments give an immediate record of the cystometric curves. But they are expensive and complex As the purpose of the study under exam ination was to show only approximately whether the Cotte Vara sympathectomy used in Finland is followed by paresis or hyper tonia of the bladder a simple water manometer was considered adequate Emmet 1954 thought that the results obtained were as satisfactory as those given by more complex apparatuses

A glass tube approximately 5 mm in diameter and fixed to a board with a graduated scale 1 5 m high was connected by a Y joint with the plastic tubes leading from the fluid transmission flask and the Nelaton catheter introduced into the bladder The graduated fluid transmission flask containing physiological saline solution at body temperature was suspended on a rack about 1 8 m high All the plastic tubes the glass bell of the fluid trans mission tubing and the glass tube serving as the manometer were of equal thickness and transparent First air was expelled from the tubing and the bladder was emptied The zero point of the glass tube manometer was placed at the height of the patient s symphysis with the patient supine in the gynecological position Physiological saline solution was instilled into the bladder in aliquots of 50 c. c at the rate of 100 drops per minute After

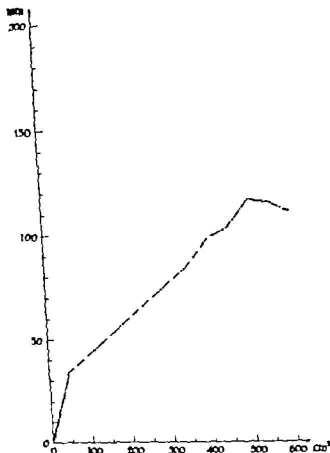


Fig. 1. Mean bladder pressure before operation

cystometry values. The feelings of filling and urinary urgency and the bladder capacity were minimal when cystometry was performed 8 days postoperatively and then rose again later. An average of 1 year 0 months after the operation the values were lower than the initial values. The micturition pressure figures also suggest hypertonicity of the bladder. As the bladder was hypertonic on straining it was incapable of raising the pressure above the mean of 41 cm. When the sympathectomy effect

Table I *Effect of Cotte-Vara Sympathectomy on the Cystometry Values*

Time of the cystometry in relation to the operation	Mean fluid volume c c				Micturition pressure in cm
	Feeling of filling of the bladder	Urinary urgency	Intensive urinary urgency	Difficulty in holding urine	
Before operation (Fig I)	159	285	450	521	60
An average of 8 days after operation (Fig II)	179	247	343	433	41
An average of 1 year 10 months after operation (Fig. III)	150	261	375	450	57

Results and Discussion

Bladder pressure curves were drawn for the cystometric bladder pressure values. The mean value curves I–IV rose least before surgery and shifted to the left postoperatively The rise was steepest 8 days after the operation and then declined gradually But the pressure of the urinary bladder was distinctly higher after subtotal hypogastric sympathectomy than before the opera tion

The shaded areas in Figs I–III denote the probability limits of the mean bladder pressure The cystometric values of the post operative measurements are within the same pressure range up to 200 c c *Thereafter the early postoperative curve continues* to climb steeply the later postoperative curve after a fall in pressure at 250 c c rises more irregularly Only 2 patients had mild bladder infection following surgery but it did not affect the form of the pressure curve

The patients noted no subjective changes in the urinary bladder after subtotal hypogastric sympathectomy Examination however revealed hypertonia of the bladder (Table I) in the postoperative

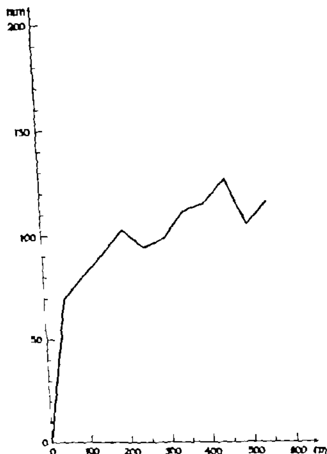


Fig. III. Mean bladder pressure an average of year 1 months postoperatively

SUMMARY

The material consisted of 30 patients in whom subtotal hypogastric sympathectomy was performed in 1959–1964 in connection with reparative surgery for primary (26/30) or secondary (4/30) sterility.

The mean age of the series was 30 1/2 years. Cystometry was performed with a simple water manometer.

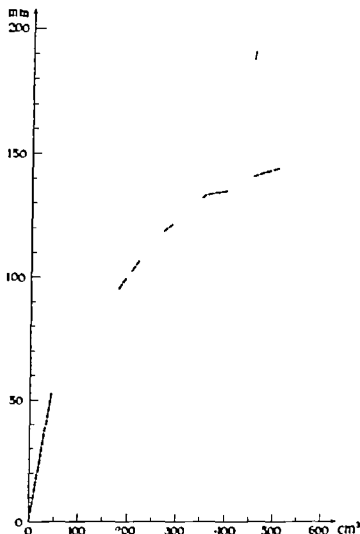


Fig II Mean bladder pressure an average of 8 days postoperatively

diminished in the course of the years the pressure again rose to 57 cm

The values recorded could not always have reflected the true situation because they were based on sensations reported by the patients themselves and the feeling of urinary urgency is also affected by the nervous condition of the patient. However the potential error must be considered to be equally great before and after the operation in each individual case

distinct shift to the left was noted. The bladder pressure values fell somewhat in the course of the years.

Subjectively the patients observed no change in the bladder but examination revealed bladder hypertonia in the postoperative cystometric values.

Acknowledgement

The author wishes to thank Professor Vara for making the patients available and Mr Martti Kaila Ph. D for doing the probability calculations for the mean value curves.

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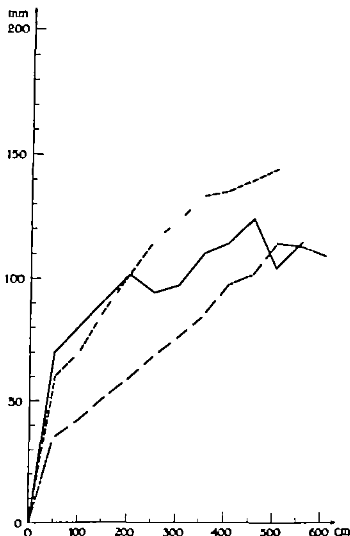


Fig. IV Mean bladder pressure before operation (– –) 8 days ( ) and 1 year 10 months after operation (———)

The first urinary bladder pressure measurement was made on the day before the operation, the second an average of 8 days after the operation and the third an average of 1 year 10 months postoperatively Bladder adaption values were considerably lower than the first values after instillation

The bladder pressure curve was most even before the opera tion and steepest at the first postoperative cystometry when a

Holmberg, Nils Gunnar. Acta obst. et gynec. scandinav. 45, 377, 1966

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CLINICAL EVALUATION OF THE PELVIC OUTLET

BY

NILS GUNNAR HOLMBERG

The prevention of complications arising during labour from the unexpected presence of a narrow pelvis is still a major point of interest in obstetrics.

Narrow pelvic inlets and gravely deformed pelves have become increasingly rare in Sweden due principally to higher standards of living in the majority of people. Complications arising from these types of pelvis malformation become evident early in labour and thus seldom present any major problems in diagnosis. Narrow outlets, however, are still quite common, their frequency being given by Borell and Fernström (1960) as 0.2–2.5 per cent and by Williams (1941) as 6 per cent. They thus constitute a problem not infrequently met with in a delivery ward. Furthermore, they cause difficulties late in labour, often associated with an emergency operation which may be made more difficult and dangerous than expected both for the mother and the child. An early diagnosis of outlet contraction, by routine antenatal evaluation of the pelvis, is therefore of importance.

The only exact method of assessing pelvic dimensions is by X-ray pelvimetry, but obviously it is possible to examine only a limited number of patients in this way. It is therefore necessary to use other methods to get at least an approximate assessment of the pelvic outlet. Most textbooks of obstetrics contain descriptions of different methods of digital examination, but it is doubtful whether the dimensions determined by these methods are sufficiently accurate to serve as a basis for clinical evaluation.

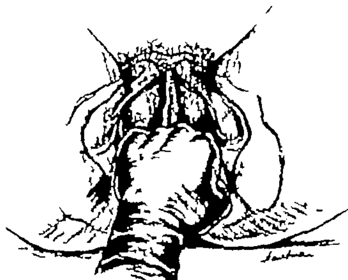


Fig Measuring bituberal diameter with fist. (From Greenhill, J P Obstetrics Ed. Philadelphia, W B Saunders Company 960)



Fig 2 Measuring sagittal outlet diameter by vaginal examination (From Thoms H Pel imetry New York Paul B Hober Inc. 956)

The aim of the investigation reported here was to see whether this is the case.

Among other investigators Borell and Fernström (1960) in an analysis of a series of 3200 X-ray pelvimetry examinations from 38 000 deliveries showed that the measurement of only one diameter of the outlet is not sufficient On the other hand, the sum of the intertuberous and the sagittal outlet diameters gives a good idea of the capacity of the outlet and if to the sum of these the interspinous diameter is also added the accuracy is still greater They also showed that there is a very close correlation between the intertuberous and interspinous diameters Their comparison of different diameters and the outcome of labour furthermore shows that a sum of these three diameters exceeding 31 5 cm is adequate for all normal-sized children (Borell and Fernström 1960 Borell and Rådberg, 1964) A sum below 29 5 cm on the other hand, very frequently causes grave disproportion and is therefore considered inadequate. When the sum of the diameters lies between 31 5–29 5 cm complications may or may not arise This thus constitutes a border-line group where individual evaluation is essential.

Method and Material

In order to apply the above findings to the routine examination of an obstetrical patient, it was decided to measure manually two diameters the intertuberous and the sagittal outlet. The limits of both these diameters are comparatively easy to palpate The intertuberous diameter was determined by a method described by Greenhill (1956) among others, whereby the clenched fist of the examiner is placed between the ischial tuberosities (Fig. 1) Assuming a normal hand's breadth of 8–9 cm and a thickness of the soft parts of 1–1 5 cm an intertuberous diameter of 10 cm is necessary to allow the fist to pass between the tuberosities. This figure is considered normal (Greenhill 1955 Thoms, 1956 and others) —The sagittal outlet diameter was determined by Thoms (1956) method wherein during vaginal examination the tip of the index finger is placed on the sacrococcygeal joint and the base of the finger

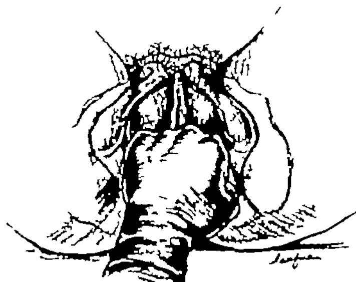


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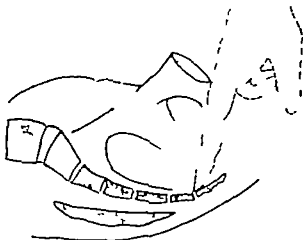


Fig Measuring sagittal outlet diameter by vaginal examination (From Thoms, H Pelvimetry New York Paul B Hober Inc. 958)

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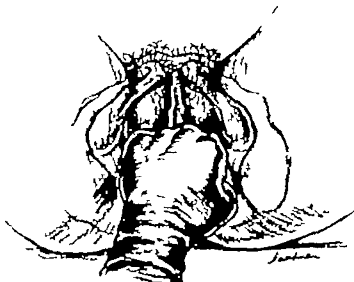


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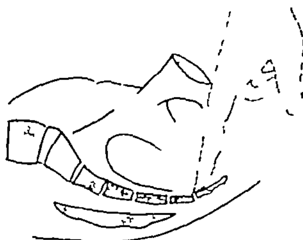


Fig Measuring sagittal outlet diameter by vaginal examination (From Thoms, H Pelvimetry New York Paul B Hoeber Inc 956)

against the lower edge of the symphysis. The intervening distance is measured directly and should normally be about 11 cm (Klein 1896 Thoms 1956)

Owing to the fact that these examinations only give an approximate estimate of the diameters it was considered preferable to use the individual measurements rather than their sum when assessing normality

The series consists of 557 primigravidæ who were admitted to the delivery ward of Karolinska Sjukhuset during Jan.–Oct., 1963 All were examined by the author usually immediately after admission. Those who showed diameters below normal *i e* an intertuberous diameter less than 10 cm and/or a sagittal outlet diameter less than 11 cm were submitted to X-ray pelvimetry For comparative purposes patients referred to the X-ray Dept. for other reasons and who had been found normal at the preliminary examination were studied. X-ray pelvimetry was performed and evaluated according to the method and standards of Borell and Rådberg (1964)

Results

As is shown in Table I, 24 cases out of 557 *i e* 4.3 per cent, were diagnosed as having an inadequate pelvic outlet and accordingly X-ray pelvimetry was carried out. The diagnosis was confirmed in 14 cases (60 per cent) and disproved in 10 cases (40 per cent) This gives a frequency of narrow outlets of 2.5 per cent. In four of these 14 cases the sum of the outlet diameters was $<$ 29.5 cm. The remaining 10 cases belonged to the borderline group

Forty-eight women where the outlet was considered normal, were X-rayed from other reasons. In all cases the X-ray pelvimetry confirmed the preliminary finding of a normal outlet

In the remaining 485 women who showed a clinically normal outlet and who were not referred for X-ray examination, there was nothing in the course of labour that pointed to the presence of outlet contraction.

Table I. *Comparison of Results of Clinical and X-ray Pelvimetry*

	Palp. Narrow X-Ray Normal	Palp. Narrow X-Ray	Total Palp. Narrow	Palp. Normal X-Ray	Palp. Normal X-Ray Narrow	Palp. Normal Labour	Total
No.	0	14	24	48		485	557
%	1.8	2.5	4.3	8.6		87.2	00.0

Discussion

The results of the investigation show that all cases of outlet contraction were diagnosed correctly i.e. there are no false negatives. On the other hand, the number of "false positives" 40 per cent, seems rather large. As most of these cases occurred at the beginning of the series the errors might in part be ascribed to lack of experience with the method. It might also be explained partly by obesity of the patient or by a tendency to an increased inclination of the pelvis causing the examiner's fist to be placed too high in the pubic arch. To avoid this last pitfall the examination should be carried out at least in doubtful cases with the patient in exaggerated lithotomy position. In this way it is also possible to get a more correct conception of the sagittal outlet diameter which thus may increase by 1-2 cm (Borell and Fernstrom, 1957)

There is a possibility that a small interspinous diameter might co-exist with a normal intertuberous diameter. However this is exceptional, as has been mentioned above but it might be pointed out that all cases of mid-pelvic arrest of the foetal head should be re-evaluated by X-ray pelvimetry even in the presence of normal intertuberous and sagittal outlet diameters.

Conclusions

1. Apart from the above objections, it seems evident that the method described is sufficiently exact to allow its use in diagnostic obstetrics.
2. If it is used routinely no case of outlet contraction need pass unnoticed.

3 Owing to its simplicity it can very easily be utilized as a screening test at centres for antenatal care. In consequence about 3–4 per cent of all primigravidæ attending such a centre would be referred for X ray pelvimetry in the last month of pregnancy As has been postulated repeatedly (e g Borell *et al*, 1958) the supposed risk caused by ionising radiation is so small with a correctly performed X ray pelvimetry that it is more than countered by the advantages gained by the obstetrician which will help him to avoid complications and to bring about safer deliveries

SUMMARY

Five hundred and fifty-seven primigravidæ were examined by a combined palpatory method in order to evaluate the pelvic outlet. 24 doubtful cases were referred for X ray pelvimetry Diagnosis of outlet contraction was confirmed in 14 cases and disproved in 10 cases No false negative results were obtained.—The reliability of the method is discussed and its use in antenatal care is pointed out.

Acknowledgement

My thanks are due to Dr J P Greenhill and W B Saunders Co and to Dr H Thoms and Paul B Hober Inc for their kind permissions to use the illustrations in figs 1 and 2

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Received on March 30th 1966

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From the Institute of Midwifery (Professor Heikki Pulkkinen and K. E. U. Jäämeri M.D.) Helsinki Finland

ON SHIVERING IN ASSOCIATION WITH NORMAL DELIVERY

BY

K.E.U. JÄÄMERI, ALARIK JAHKOLA AND JORMA PERTTU

It is well known by all obstetricians that a certain number of their parturients present a shivering phenomenon during or more often, immediately after childbirth. This phenomenon once mentioned in the old textbooks is now practically forgotten. This is understandable owing to the lack of clinical importance of this symptom, at least as judged by our present outlook. However a few years ago Bruniquel (1963) drew attention to the post partum shivering of mothers He considered several possible explanations for this and finally came to the conclusion that the most plausible pathogenic factor would be penetration of amniotic fluid into the maternal circulation during labour

In order to establish the correlations of this phenomenon we have made a note of it in delivery reports in our clinic since 1964 and have now analysed a series of a thousand normal childbirths in this respect

The series comprises 500 shivering patients compared with 500 controls All pathological cases were excluded, but otherwise the patients were taken without selection in order of admission to the clinic until the number of cases in both groups reached 500 When this figure was reached in the control group there were 147 shiverers which means that the general occurrence of shivering associated with a normal confinement was 22 7 per cent. Out of the complete group of 500 shiverers 22.4 per cent were classified as severe tremblers. The data was processed by com

Table I *The Correlation of Shivering with Different Factors Connected with Delivery*

	Shivering Group 500	Controls 500	Severe tremblers
Age in years-mean	26.5 +++	24 4 +++	27 5
Parity mean	2.0	1 9 +	2.1
Multiparas	57.4 %	54 8 % +	66 6 %
Unmarried	7.8 %	5.4 %	1.0 %
Blood group A	44.0 %	41 6 %	43 0 %
" B	14 0 %	18.2 %	16.0 %
" O	32.4 %	31.2 %	30.3 %
" " AB	9.6 %	9 0 %	10.7 %
" " RH+	93 0 % +	87.4 %	86 6 %
Duration of the gestation-mean	5	5	5
Nutritional state			
Normal	81.4 %	85.2 % ++	74.1 %
Obese	12.4 %	12.0 %	17 9 %
Thin	6.2 % ++	2.8 % ++	8.0 %
Accelerated respiration	1.0 %	0	0.6
Sensitive disposition	4.0 % +	1 8 % +	5.3 %
Quant. of amniotic fluid			
Normal	81.2 % +++	89.4 % +++	75.9 %
Abundant	6 4 %	5.6 %	7 1 %
Scanty	12.4 % +++	5.0 % +++	7 0 %

putor and analyses included the t test for statistical significance The results are presented in detail in Tables I–IV The level of statistical significance is expressed in the tables as follows

+ level of significance	95–99	per cent
++	99–99 9	
+++	over 99 9	

Once in possession of the basic answers from our investigation we decided to separate the group of severe tremblers (112) in order to ascertain which of the primary differences could be confirmed in this way The third column of Tables I–III shows the values for the severe tremblers and this mainly confirms the

Table II. The Correlation of Shivering with Different Factors Connected with Delivery

	Shivering group 500	Control group 500	Severe tremblers 13
Duration of labour-mean	5 h	15.2 h	14 h
Stage I-mean	4.8 h	14.7 h	3.7 h
II-	0.3 h	3 h	0.3 h
III-	0.15 h	0.13 h	0.13 h
Uterine contractions			
Normal	72 %	74 %	70.6 %
Strong	21.4 %	20.6 %	20.5 %
Weak	6.6 %	5.4 %	8.9 %
Rupt. of the membranes			
Before	8.7 %	17.6 %	20.5 %
Stage I	73.7 %	73 %	71.4 %
II	5.6 % +	9 %	8.1 %
Haemorrhage	272 g	46.5 g	135.2 g
Ragged placenta	36.2 %	37 %	33.0 %
Perineal tear or episiot	52.8 %	58.4 %	57.1 %
Twisted umbilical cord	34.2 %	34 %	43 %
Temperature after deliv -mean	36.9	36.9	37.0
Pulse rate -mean	79.4/min.	74.3/min.	75.0/min
Proteinuria -mean	4 %	6.2 %	5 %

previous results. We were also interested to know if the most striking differences statistically namely maternal age and scanty amniotic fluid, could possibly be interdependent without any connection with the shivering. No direct correlation between the two factors was evident from the computor data.

Discussion

The first factor maternal age revealed a highly significant difference (over 99.9 per cent) This could be partly explained at least by the fact that multiparas are probably more prone to this symptom which again could indicate a previous sensitization if we accept the theory of amniotic embolism. On the other hand

Table III. *The Correlation of Shivering with Different Factors Connected with Delivery*

	Shivering group 500	Control group 500	Severe tremblers 112
Weight of the baby-mean	3.5 kg	3.5 kg	3.5 kg
Male child	51.0 %	47.4 %	54.6 %
Asphyxia	1.8 %	1.2 %	1.8 %
Apgar score-mean	9.5	9.4	9.5
Genital infections	8.0 %	5.4 %	7.0 %
Lochia			
Normal	92.8 %	92.5 %	95.5 %
Abundant	1.8 %	1.6 %	1.8 %
Scanty	5.4 %	5.9 %	2.7 %
Subinvolution of the uterus	1.4 %	1.0 %	0.0 %
Peripheral thrombosis	0.2 % +	1.2 %	0.0 %
Cough medicine	4.6 % +	2.2 % ++	7.1 %

Table IV. *The Frequency of Shivering in the Different Stages of the Delivery*

	Shivering group	Severe tremblers
Delivery	500	112
Stage I	42.4 %	52.7 %
„ II	16.2 %	-6.8 %
„ III	23.2 %	35.7 %
Later	59.4 %	62.5 %

the number of previous deliveries seemed to be without significance. The common maternal blood groups, including Rh, did not reveal any differences in distribution. Unfortunately it was not possible to obtain the blood group of all the children. The duration of gestation in the two groups was absolutely identical. We accepted cases from 4 weeks before to 4 weeks after the calculated time of birth and these periods are designated 1 (36 weeks gestation) — 9 (44 weeks gestation). Fig. 5 thus denotes a term pregnancy. A subnormal body weight seems to predispose to shuddering, as does a sensitive disposition. The latter connec

tion is readily understandable. The former again could point to an asthenic constitution with perhaps more friable tissues the quality of the membranes and placental tissue might have some importance as a pathologic factor with reference to the hypothesis of leak of amniotic fluid into the maternal circulation. Accelerated respiration (hyperventilation) was without importance.

The most interesting finding was that the shivering group showed a highly significant increase in the proportion of patients with scanty amniotic fluid. Could this correlate with the theory of amniotic embolism as a possible pathogenic factor? Contrary to expectations neither the duration of labour the intensity of the uterine contractions nor the time of rupture of the membranes had any significance The absence of hæmorrhage or elevation of temperature or pulse rate directly after delivery probably indicate that in these cases the assumed penetration of amniotic fluid into the uterine veins was not profuse enough to produce any of these reactions The occurrence of perineal tears, a rugged placentæ and twisted umbilical cords was the same in both groups.

The weight or condition of the child did not influence the symptom in question but the slight preponderance of male children in the shivering group is interesting, though not statistically significant. Furthermore genital infections peripheral thrombosis, quantity of lochia and uterine atony (subinvolution) can be excluded as factors The last factor recorded cough medicine was an attempt to detect pulmonary complications after an assumed amniotic embolism because checking the lungs of every puerperal patient has not been routine procedure at our clinic Patients with bronchitis were of course excluded with other pathological cases, from this material. A certain difference might be established in this way

Regarding the shivering itself we can see that the emphasis came in the immediate post-partum period and also that the heavy tremblers had a tendency to shiver throughout the delivery

To conclude in evaluating the importance of an amniotic embolism in this connection, it is necessary to recall certain facts. It has been established quite recently that the amniotic proteins are of

maternal origin (Seppälä, 1966) On the other hand, we must bear in mind the finding of trophoblastic elements in the maternal circulation (Douglas *et al* 1959 Jäämeri *et al.* 1965) and their intrusion might be partly coincident with an amniotic leak. Antibodies to trophoblasts have also been found, principally during the post-partum period (Hulka *et al.* 1963) As a third occurrence we have still to remember the penetration of fœtal blood into the maternal veins. For example the percentages of this phenomenon given by Verdir 1966 24 per cent before and 27 8 per cent after delivery are well in accordance with our incidence of shivering Finally we think that there are quite a number of slight detachments of the placenta which remain un diagnosed and are therefore included among the normal cases.

SUMMARY

The authors have presented the results of their investigation concerning the neglected obstetrical phenomenon of shivering associated with normal delivery A series of 1000 cases has been analysed with special reference to hypothetic amniotic embolism.

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INFLUENCE OF HEREDITY AND ENVIRONMENT ON NORMAL MENSTRUAL BLOOD LOSS

A Study of Twins

BY

GÖRAN RYBO AND LEIF HALLBERG

Biological variations in man are very often caused by an interaction between hereditary and environmental factors. Some data suggest that heredity influences the magnitude of the menstrual blood loss. Thus, in a previous study it was found that there was a pronounced individual constancy in menstrual blood loss between different women (H a l l b e r g and N i l s s o n, 1964 a). A hereditary factor may explain such an individual constancy. Furthermore, L u n d h o l m (1939, 1959) found that in families in which one member had hypochromic anæmia there was a great risk that younger sisters above the age of 20 would develop the same disease. However, this risk was much lower for younger brothers, and also for females above the age of 50. The author assumed that a dominant gene may cause an inadequate absorption of iron, which is revealed when iron is lost by hæmorrhage. However, heavy menstrual blood loss is probably a main reason for the development of iron deficiency in women, (H a l l b e r g, H ö g d a h l, N i l s s o n, and R y b o, 1966 a). A hereditary influence on the menstrual blood loss may thus be reflected by variations in the iron balance which are falsely interpreted as primarily hereditary.

Further support for a hereditary influence on the magnitude of the menstrual blood loss is given by the finding that in patients with heavy menstrual blood loss there is often a higher concentration of plasminogen activators in the endometrium than in women with a menstrual blood loss within the normal range (R y b o, 1966). This observation suggests that an enzymatic

factor influences the menstrual blood loss. Such a factor might be genetically determined. Finally in clinical practice, anamnestic information sometimes indicates a familial incidence of heavy menstrual blood loss

Studies of twins make it possible to find out if a genetic component affects significantly the variation observed in a trait. Monozygotic twins contain identical genetic material, and the intrapair differences observed must be due to environmental factors Dizygotic twins however vary in their genetic constitution to the same extent as non-twin siblings and the intrapair differences are due to hereditary as well as environmental influences. Furthermore studies of twins permit an evaluation of certain environmental factors which can be clearly defined. Parity is one such well definable factor which was investigated in the present study as women sometimes state that their menstrual blood loss changed after childbirth

The aim of the present investigation was to study

1 the menstrual blood loss and its variations in monozygotic and dizygotic twins in order to investigate if there is a genetic component contributing to the variations and

2 the effect of parity as one possible environmental factor

MATERIAL

The twin sample was obtained from the register of twin births in the Department of Obstetrics and Gynæcology Göteborg, between 1925 and 1945 This period was chosen to avoid any influence of menarche and menopause Most deliveries in the city during this period took place in hospitals and more than half of them in the Department of Obstetrics and Gynecology During the 20-year period mentioned above 231 live female twin pairs were born At the time of the investigation (January–April 1965) 125 of these pairs were still living in or near the city 71 pairs were prepared to participate in the study but 16 of these had to be excluded for the following reasons

In 8 pairs one or both twins had amenorrhœa due to pregnancy

In 5 pairs one or both twins used oral contraceptives which

influence the menstrual blood loss (Hallberg, Nilsson and Rybo 1964, Nilsson and Rybo 1966).

In 2 pairs, one or both twins refused subsequentely to measure their menstrual blood loss as they found it difficult to collect sanitary towels and tampons at their working places.

In one pair the zygosity could not be established.

In the remaining 55 pairs the menstrual blood losses were measured in both twins. A general examination showed that all subjects were healthy except for one who had well controlled diabetes mellitus. 25 pairs were diagnosed as monozygotic (MZ) and 30 pairs as dizygotic (DZ) twins.

In a previous study on the menstrual blood loss in a large population sample, it was concluded that a blood loss exceeding 80 ml per period does not fall within the limits of the normal variation (Hallberg, Högdahl, Nilsson, and Rybo 1966 a). In the present series 3 MZ and 4 DZ twins had a menstrual blood loss above this value although their cotwins had values within the normal range. Studies on the genetic influence on continuous variables using the twin method must usually be restricted to normal subjects. Consequently these 7 pairs were excluded. The number is too small to study the influence of hereditary and environmental factors in menorrhagia. Data for each of the remaining 22 MZ and 26 DZ pairs is given in the Appendix.

The statistical analysis used in this study are based upon two menstrual measurements in each subject. However, in 6 pairs (4 MZ and 2 DZ) only a single measurement was made in one or both twins. These pairs had to be excluded from the statistical analyses. Thus, the final series comprised 18 MZ and 24 DZ pairs.

The mean age of the MZ twins was 27.8 ± 1.4 years (range 19–38) and of the DZ twins 27.8 ± 1.6 years (range 19–38). The age and parity distributions are shown in Table I. There were no systematic differences between MZ and DZ twins with respect to age and parity.

Subdivisions were made to obtain groups of MZ and DZ twins which were similar or dissimilar with respect to parity. The menstrual blood loss and its variation was studied in the

factor influences the menstrual blood loss. Such a factor might be genetically determined Finally in clinical practice anamnestic information sometimes indicates a familial incidence of heavy menstrual blood loss.

Studies of twins make it possible to find out if a genetic component affects significantly the variation observed in a trait. Monozygotic twins contain identical genetic material and the intrapair differences observed must be due to environmental factors. Dizygotic twins however vary in their genetic constitution to the same extent as non twin siblings and the intrapair differences are due to hereditary as well as environmental influences. Furthermore studies of twins permit an evaluation of certain environmental factors which can be clearly defined. Parity is one such well definable factor which was investigated in the present study as women sometimes state that their menstrual blood loss changed after childbirth.

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In 8 pairs one or both twins had amenorrhœa due to pregnancy

In 5 pairs, one or both twins used oral contraceptives which

had one or more children or in which the twins had different number of children ("the unequal-parity group")

3. Pairs in which both twins were nulliparous ("the nulliparous group")

4. Pairs in which one twin was nulliparous and the cotwin had one or more children ("the nulliparous-parous group")

The number of subjects and the mean age of each subgroup are given in Table II and the distribution of parity in Table III.

METHODS

A letter was sent to the twins stating the purpose of the study. Furthermore they were offered a free health screening. The examinations included a detailed medical and menstrual history. A venous blood sample was drawn to determine sedimentation rate, haemoglobin concentration, mean corpuscular haemoglobin concentration, plasma iron concentration and total iron binding capacity in serum. The urine was tested for albumin and glucose. The results of some of these analyses will be published separately. Each woman was examined gynaecologically and a vaginal smear was subjected to microscopy. However no pathological gynaecological findings of importance were observed in any of the subjects.

1 *Laboratory methods*

The determinations of the menstrual blood loss were made according to a method described by Hallberg and Nilsson (1964 b). Detailed instructions were given to all women to use tampons and towels simultaneously when collecting the menstrual blood to reduce the waste of blood as much as possible. The mean value of two consecutive periods was used as a measure of the individual menstrual blood loss. The haemoglobin was determined in venous blood as cyanmethaemoglobin. The menstrual blood loss was calculated in ml.

2 *Zygosity classification*

The zygosity classification was made mainly by a questionnaire method described and analysed by Cederlöf Friberg, Jons-

Table I. *Distribution of Age and Parity*

	Age[1]			Parity			
	9–5	26–32	33–39				>
MZ	8	5	5	19	9	3	5
	Mean 27.8±1.4						
DZ	10	7	7	29	8	10	1
	Mean 27.8±1 6						

For age the numbers given are equal to pairs, for parity the numbers refer to subjects.

Table II. *Number of Pairs and Mean Age in the Various Subgroups*

	Equal Parity		Unequal Parity		Nulliparous		Nulliparous–parous	
	Mean age ± standard error of mean	Number of pairs	Mean age ± standard error of mean	Number of pairs	Mean age ± standard error of mean	Number of pairs	Mean age ± standard error of mean	Number of pairs
MZ	24 9±2.1	10	31 5±1 9	8	21.0±0.6	7	29.4±2.5	5
DZ	26.4±1 7	16	30.4±2.2	8	23 1±1 1	11	29 9±2.5	7

Table III. *Distribution of Parity within Pairs*

	Equal Parity					Unequal Parity							
	0/0[1]	/	/2	3/3	Total	0/	0/2	0/3	/	/3	/3	/5	Total
MZ	7	2	0	1	10	3	1	1	1	1	1	0	8
DZ	11	2	3	0	16	4	3	0	0	0	0	1	8

The ratios show the number of children for the twins within a pair

entire series and in the subgroups. Studies on the subgroups made it possible to investigate the influence of parity as an environmental factor. The following subgroups were studied.

1. Pairs in which both twins were nulliparous or had an equal number of childbirths ("the equal-parity group")

2. Pairs in which one twin was nulliparous and the cotwin

had one or more children or in which the twins had different number of children ("the unequal-parity group")

3. Pairs in which both twins were nulliparous ("the nulliparous group")

4. Pairs in which one twin was nulliparous and the cotwin had one or more children ("the nulliparous-parous group")

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2. *Zygosity classification*

The zygosity classification was made mainly by a questionnaire method described and analysed by Cederlöf Friberg, Jone-

son and Kaij (1961) Four pairs in the final series could not be classified by this method. In 3 of these the zygosity was established later on by means of blood group examinations in the Blood Group Laboratory comprising 5 independent systems, namely ABO Rh (complete genotype determination) K, Fy and MN

The questionnaire was sent to the twins at the same time as the introductory letter The zygosity classification was based on the answers to the following questions

1 Were you and your twin sister mixed up as children? If yes by whom? Parents? Brothers and sisters? Teachers? Other people?

2 When growing up were you and your twin sister "as like as two peas" or only of ordinary family likeness?

Furthermore the twins were asked if they lived together or not at the time of the examination and if separated, until what age they lived together

Before a twin pair was classified as monozygotic the following criteria had to be fulfilled *Both* twins had to reply in the questionnaire that they were as like as two peas *and* that they had been mixed up as children. These criteria were fulfilled by 16 out of 18 MZ pairs in the final series In the remaining 2 pairs the twins gave contradictory answers to one of the questions. The zygosity was then established by means of blood group examinations To classify the twins as dizygotic *both* twins had to reply that they had not been mixed up *and* that they were of ordinary family likeness. These criteria were fulfilled in 25 out of 26 DZ pairs in the final series. In the remaining pair included in the DZ group both twins answered that they had not been mixed up but the answers to the question about likeness in the questionnaire were lacking in both twins. However on a direct question about likeness they answered that they were of ordinary family likeness only Furthermore this pair was classified as dizygotic on account of disconcordance of blood groups.

In one excluded pair blood samples for blood group examinations could not be obtained because one of the twins committed suicide

3. *Statistics*

The arithmetic mean and its standard error were calculated according to conventional methods. The significance of differences between mean values was tested by Student's t test.

In studies of twins certain statistical methods are generally used *e.g.* analysis of variance and calculation of intraclass correlation coefficients. The present study also comprised an investigation of the individual variation by measuring the menstrual blood loss at two consecutive periods. The individual variation was thus accounted for in the statistical analyses of variance. The calculations were made according to conventional formulas for a hierarchial analysis of variance (see *e.g.* Brownlee 1961)

The following variances were calculated

Variance between measurements within individuals for MZ and DZ twins respectively ($V_{wm/mz}$ and $V_{wm/dz}$)

The intrapair variance *e.g.* the variance between individuals within pairs (V_{mz} and V_{dz}) This variance was calculated from the mean values of the two menstrual measurements in each subject and had to be multiplied by two to make it comparable with the variance between measurements.

The interpair variance *e.g.* the variance between pairs (V_{p}) This ariance was calculated for the DZ group only In each pair the means of the two measurements for each twin were used for the calculation of the average value for the pair This variance had to be multiplied by four to make it comparable with the intrapair variance

The following ratios were calculated and tested by a one-tailed F-test

V_{mz} $V_{wm/mz}$ Statistical significance indicates a real influence of environment.

V_{dz} $V_{wm/dz}$ Statistical significance indicates a real influence of heredity and/or environment.

V_{dz} V_{mz} Statistical significance indicates a real influence of heredity

V_{p} V_{dz} which gives an estimate of the relation between unrelated subjects and dizygotic twins. Statistical significance in-

dicates an influence of further environmental and/or hereditary factors.

In addition to these calculations the intraclass correlation coefficient for MZ (r_{mz}) and for DZ (r_{dz}) was calculated according to the formula $r = 1 - \frac{V}{2s^2}$ (Fischer 1948) where V is the intrapair variance and s^2 the variance of all measurements (mean value of two consecutive periods) within the group. The higher the intrapair variance compared with the variance of all the individual mean values within the group, the lower is the intraclass correlation coefficient. The significance of the difference between intraclass correlation coefficients was tested by Fisher's z-transformation test.

RESULTS

1 *Entire series*

The results are shown in Table IV. The mean value of the menstrual blood loss was slightly lower in the MZ group than in the DZ group but the difference was not statistically significant. The mean difference between periods was almost the same for the DZ twins and for the MZ twins. Table IV also shows that the ratio V_{mz} $V_{bm/mz}$ was statistically significant. This indicates that environmental factors influence menstrual blood loss. The ratio V_{dz} $V_{bm/dz}$ was also statistically significant, indicating an influence of heredity and/or environment. The ratio V_{tp} V_{dz} was not statistically significant, which implies that the menstrual blood loss varies between sisters to the same extent as in unrelated women. The statistically significant F value for the ratio V_{dz} V_{mz} indicates a hereditary influence on the menstrual blood loss. This is evident also from the difference between the intraclass correlation coefficients, which was higher in MZ twins than in DZ twins. The difference was statistically significant ($Z = 1.99$, $p < 0.05$).

2 *The equal parity group*

The equal-parity group included also those pairs in which both twins were nulliparous. The results, shown in Table V, are

Table IV Statistical Analysis of Entire Series

	MZ	N	DZ	N
Mean value (ml)	26.8 ± 2.00	36	29.3 ± 2.21	48
Mean difference between periods (ml)	5.9 ± 1.15	36	6.2 ± .68	48
Mean difference within pairs (ml)	6.8 ± .78	18	14.6 ± 3.05	24
Intraclass correlation coefficient	0.64		0.07	

Analysis of Variance

Variance and ratios	MZ	DZ	F-value	P
V_{bm}	46.30	45.18		
V	0 .02	427.74		
V_{ip}		470.24		
V_{mz} $V_{bm/mz}$			2.18	<0.025
V_{dz} $V_{bm/dz}$			9.47	<0.0005
V_{dz} V_{mz}			4.23	<0.005
V_{ip} V_{dz}				not sign.

essentially in agreement with those obtained when the entire series was considered. However in this group the concordance within MZ and DZ pairs is higher than in the entire series. Thus, r_{mz} increased from 0.64 in the entire series to 0.76 in the equal-parity group. The corresponding increase for r_{dz} was from 0.07 to 0.32 The ratio V_{mz} $V_{bm/mz}$, however was not significant in this group, which may indicate a reduced variation in environmental influence within pairs. The interpair variance (V_{ip}) is not given for this group since the parity was not the same between pairs as within pairs.

3 The unequal parity group

The results for this group are shown in Table VI. In contrast to the equal-parity group the ratio V_{mz} $V_{bm/mz}$ was significant. This may be due to a greater variation in environmental in-

Table V *Statistical Analysis of the Equal Parity Group*

	MZ	N	DZ	N
Mean value (ml)	8 6±2 70	0	77 7±2 53	32
Mean difference between periods (ml)	5 4±1 48	20	7 5±1 19	32
Mean difference within pairs (ml)	6.2±1 92	10	12.2±3.00	16
Intraclass correlation coefficients	0 76		0.32	

Analysis of Variance

Variance and ratio	MZ	DZ	F-value	P
V_{bm}	41 44	50 39		
V	66 10	268 62		
V_{mz} $V_{bm/mz}$			1 60	not sign.
V_{dz} $V_{bm/dz}$			5 33	<0.0005
V_{dz} V_{mz}			4 06	<0.025

fluence. The hereditary component however is clearly shown by the significant V_{dz} V_{mz} ratio The variances between measurements are of the same magnitude as in the equal-parity group for MZ and DZ twins which indicates that a difference in parity does not influence the individual variation. The V_{b} was not calculated for the reason given for the equal-parity group The correlation coefficients were significantly different ($Z=2\ 14$ $p<0\ 05$) which supports the conclusion of a hereditary influence. The r_{dz} value for the unequal-parity group was lower than for the equal parity group which may indicate that a difference in parity influences the menstrual blood loss. The difference between the r_{dz} values was however not statistically significant

4. *The nulliparous group*

In this group the influence of parity on the intrapair differences is excluded. The results are given in Table VII. The group is

Table VI. *Statistical Analysis of the Unequal Parity Group*

	MZ	N	DZ	N
Mean value (ml)	20.2±4.5	6	32.5±4.3	16
Mean difference between periods (ml)	6.4± .87	6	6.2± .52	16
Mean difference within pairs (ml)	7.7±3.40	8	9.6±7.30	8
Intraclass correlation coefficient	0.76		−0.34	

Analysis of Variance

Variance and ratio	MZ	DZ	F-value	P
V_{bw}	52.38	34.78		
V	144.68	745.08		
V_{mz} $V_{bw/mz}$			2.76	<0.05
V_{dz} $V_{bw/dz}$			.45	< .0005
V_{dz} V_{mz}			5.16	<0.025

small, but the results obtained essentially agree with those of the equal-parity group. $V_{bw/dz}$ was statistically higher than $V_{bw/mz}$ ($F=3.22$, df = 22 and 14, $p<0.05$). The V_{wp} V_{dz} ratio was not statistically significant. In this group, the interpair variance can be compared with the intrapair variance since all subjects were nulliparous. The intraclass correlation coefficients in the nulliparous group were of about the same magnitude as in the equal parity group.

5. *The nulliparous-parous group*

The results are given in Table VIII. The number of pairs in the group was small: 5 MZ and 7 DZ pairs only. However, the results were very consistent with those of the unequal-parity group. The V_{wp} was not calculated since the parity was not the same between pairs as within pairs.

The main reason for selecting the nulliparous-parous group was to make a comparison with the results obtained in the

Table V *Statistical Analysis of the Equal Parity Group*

	MZ	N	DZ	N
Mean value (ml)	28 6 ± 2 70	0	27 7 ± 2.53	32
Mean difference between periods (ml)	5.4 ± 1.48	20	7 5 ± 1 19	32
Mean difference within pairs (ml)	6.2 ± 1 92	10	12.2 ± 3 00	16
Intraclass correlation coefficients	0 76		0.32	

Analysis of Variance

Variance and ratio	MZ	DZ	F-value	P
V_{bm}	41.44	50 39		
V	66.10	268 62		
V_{mz} $V_{bm/mz}$			1.60	not sign.
V_{dz} $V_{bm/dz}$			5 33	<0.0005
V_{dz} V_{mz}			4 06	<0 025

fluence The hereditary component, however is clearly shown by the significant V_{dz} V_{mz} ratio The variances between measurements are of the same magnitude as in the equal-parity group for MZ and DZ twins which indicates that a difference in parity does not influence the individual variation The V_{w} was not calculated for the reason given for the equal-parity group The correlation coefficients were significantly different ($Z = 2\ 14$ $p < 0\ 05$) which supports the conclusion of a hereditary influence. The r_{dz} value for the unequal-parity group was lower than for the equal parity group which may indicate that a difference in parity influences the menstrual blood loss. The difference between the r_{dz} values was however not statistically significant.

4. *The nulliparous group*

In this group *the influence of parity on the intrapair differences is excluded.* The results are given in Table VII The group is

Table VI. *Statistical Analysis of the Unequal Parity Group*

	MZ	N	DZ	N
Mean value (ml)	30.2 ± 4.5	16	32.5 ± 4.31	16
Mean difference between periods (ml)	6.4 ± 1.87	6	6.2 ± 1.52	16
Mean difference within pairs (ml)	7.7 ± 3.49	8	9.6 ± 7.30	8
Intraclass correlation coefficient	.76		−0.34	

Analysis of Variance

Variances and ratios	MZ	DZ	F value	P
V_{bw}	52.38	34.78		
V	144.68	745.98		
V_{mz} : $V_{bw/mz}$			2.78	<0.05
V_{dz} : $V_{bw/dz}$			21.45	< .0005
V_{dz} : V_{mz}			5.16	<0.025

small, but the results obtained essentially agree with those of the equal-parity group. $V_{bw/dz}$ was statistically higher than $V_{bw/mz}$ ($F = 3.22$, df = 22 and 14, $p < 0.05$). The V_{wp} : V_{dz} ratio was not statistically significant. In this group the interpair variance can be compared with the intrapair variance since all subjects were nulliparous. The intraclass correlation coefficients in the nulliparous group were of about the same magnitude as in the equal parity group.

5. *The nulliparous-parous group*

The results are given in Table VIII. The number of pairs in the group was small, 5 MZ and 7 DZ pairs only. However, the results were very consistent with those of the unequal-parity group. The V_{wp} was not calculated since the parity was not the same between pairs as within pairs.

The main reason for selecting the nulliparous-parous group was to make a comparison with the results obtained in the

Table VII. *Statistical Analysis of the Nulliparous Group*

	MZ	N	DZ	N
Mean value (ml)	27.6±3.26	14	29.2±3.01	22
Mean difference between periods (ml)	4.6±1.15	14	5.9±1.91	22
Mean difference within pairs (ml)	5.1±1.93	7	11.1±3.39	11
Intraclass correlation coefficient	0.84		0.40	

Analysis of Variance

Variance and ratio	MZ	DZ	F-value	P
V_{bm}	17.[illegible]1	55.76		
V	44.52	229.42		
V_{lp}	–	265.12		
V_{mz} $V_{bm/mz}$			2.59	not sign.
V_{dz} $V_{bm/dz}$			4.11	<0.005
V_{dz} V_{mz}			5.15	<0.025
V_{lp} V_{dz}			1.16	not sign.

nulliparous group. Such a comparison shows that V_{dz} was lower in the latter group. The difference between the V_{dz} values is statistically significant ($F=3.71$, $df=7$ and 11, $p<0.05$). This indicates that there was a greater variation in environment in the nulliparous parous group than in the nulliparous group. The same interpretation may be valid for the observation that the intraclass correlation coefficients were higher for both MZ and DZ twins in the nulliparous group than in the nulliparous-parous group. However, the differences were not statistically significant.

DISCUSSION

Methodological aspects

As stated in the introduction, a study of the genetic control of a continuous variable such as the menstrual blood loss, is made preferably in a twin investigation. However, contrary to family

Table VIII. *Statistical Analysis of the Nulliparous-Parous Group*

	MZ	N	DZ	N
Mean value (ml)	18.2 ± 2.31	10	3 .2 ± 4 85	14
Mean difference between periods (ml)	4 8 ± 1 37		5 9 ± 1.63	4
Mean difference within pairs (ml)	4.2 ± .24	5	22.2 ± 8.43	7
Intraclass correlation coefficients	0.75		−0.40	

Analysis of Variance

Variances and ratios	MZ	DZ	F-value	P
V_{bet}	6 04	34 33		
V	23.68	852 34		
$V_{mz} : V_{bet}/_{mz}$			40	not sign.
$V_{dz} : V_{bet}/_{dz}$			24 83	<0 0005
$V_{dz} : V_{mz}$			36.02	<0.00

studies, studies of twins give no information on the precise mode of inheritance. Theoretically pedigree analyses might be used by investigating e.g. mothers and daughters. However this approach has many disadvantages. The great difference between a mother and a daughter as to age, parity etc will probably influence the results to a great extent. Such an influence will be still more pronounced when the mother is near the menopause and the daughter not far from menarche (Hallberg, Högdahl, Nilsson, and Rybo, 1966 a)

The twin method, however also has certain limitations which must be considered when the results are interpreted. One of the bases for comparison between MZ and DZ twins is the assumption that environmental forces which affect the intrapair differences in the two groups are similar. Among others Neel and Schull (1954) pointed out the possible influence of prenatal environmental" factors such as differences in implantation of the ova, position of the fœtus in the uterus, the manner

Table VII. *Statistical Analysis of the Nulliparous Group*

	MZ	N	DZ	N
Mean value (ml)	27.6±3.26	14	29.2±3.01	22
Mean difference between periods (ml)	4.6±1.15	14	5.9±1.91	22
Mean difference within pairs (ml)	5.1±1.93	7	11.1±3.39	11
Intraclass correlation coefficient	0.84		0.40	

Analysis of Variance

Variance and ratio	MZ	DZ	F-value	P
V_{bm}	17.21	55.76		
V	44.52	229.42		
V_{lp}	–	265.12		
V_{mz} $V_{bm}/_{mz}$			2.59	not sign.
V_{dz} $V_{bm}/_{dz}$			4.11	<0.005
V_{dz} V_{mz}			5.15	<0.025
V_{lp} V_{dz}			1.16	not sign.

nulliparous group Such a comparison shows that V_{dz} was lower in the latter group The difference between the V_{dz} values is statistically significant ($F=3.71$ $df=7$ and 11 $p<0.05$) This indicates that there was a greater variation in environment in the nulliparous-parous group than in the nulliparous group The same interpretation may be valid for the observation that the intraclass correlation coefficients were higher for both MZ and DZ twins in the nulliparous group than in the nulliparous-parous group However the differences were not statistically significant.

DISCUSSION

Methodological aspects

As stated in the introduction a study of the genetic control of a continuous variable such as the menstrual blood loss, is made preferably in a twin investigation However contrary to family

per cent) were serologically classified as monozygotic. Thus, when this method of zygosity classification is used there is a small risk that the DZ group contains MZ twins. This error will affect the results in such a way that the mean intrapair differences in MZ and DZ twins will tend to become equalized and will therefore not strengthen any positive results. Favourable results with similar criteria for zygosity classification have previously been reported by Essen-Möller (1941) Norinder (1948) and Husén (1953)

In 3 pairs, the zygosity classification could not be made according to the questionnaire method as the criterion that both questions should be answered concordantly by both twins was not fulfilled. In these pairs the zygosity classification was made by serological determinations using 5 independent systems including a complete genotype determination of the rhesus system. When such blood group determinations are used it has been estimated that the median probability of concordant pairs being monozygotic is about 96 per cent (Cederlöf Friberg, Jonsson, and Kaij, 1961)

The twin series in this investigation is not representative for the twin population as a whole because the use of volunteers was inevitable. However such a sampling bias is probably of no importance in a study of this kind. Of greater importance is the fact that the study was restricted to normal subjects. For this reason pairs in which one twin had menorrhagia were excluded.

Hereditary influence on the menstrual blood loss

The results obtained in the entire series as well as in the various subgroups show that there is a hereditary influence on the magnitude of the menstrual blood loss. This is evident from the statistically significant ratio V[illegible] V[illegible] and from the differences between the intraclass correlation coefficients in the MZ and DZ groups The ratio V[illegible] V[illegible] was statistically significant in the entire series which indicates that there is also an environmental influence on the menstrual blood loss. The significant V[illegible] V[illegible] ratio may thus indicate that heredity *and* environment influence the menstrual blood loss.

of delivery etc which may be reasons for non-genetic differences Differences in the environmental influence may also occur in post-natal life MZ twins are often inseparable, often dress alike similarities in behaviour are expected of them, and they tend to identify themselves with one another DZ twins however are usually exposed to a more differentiated external environment in these respects. Obviously these environmental differences between MZ and DZ twins may be of importance when psychological factors are studied The significance of these differences with respect to a variable such as the menstrual blood loss is very difficult to assess. There is no reason to believe however that environmental differences such as the ones mentioned above should be of any significance as far as the menstrual blood loss is concerned

One factor in the present study which may affect the intra pair differences in various ways in MZ and DZ twins is the similarity or dissimilarity in the collection technique between the twins when towels and tampons were used. MZ twins are probably more concordant in their technique than DZ twins. There is no reason to believe however that MZ twins in general are more or less careful than DZ twins The small error inherent in the present collection technique always means a waste of blood However the mean values of the menstrual blood loss in the two groups of twins were not significantly different indicating that there was no systematic difference in collection technique between MZ and DZ twins Furthermore judging from the twins own statements as to waste of blood during the collection, such a waste must be considered small To some extent this is also supported by the agreement between the two consecutive measurements in both MZ and DZ twins.

Another error of the twin method is related to the zygosity classification (see *e.g* Walker 1957) In the present study most pairs were classified according to the questionnaire method used by Cederlof Friberg, Jonsson and Kaij (1961) These authors found that when both twins replied as like as two peas *and* mixed up all were monozygotic also according to serological determinations. When both twins replied only family likeness *and* not mixed up 9 out of 108 pairs (8 3

parity and menstrual blood loss. Further studies, designed in some other manner are necessary to elucidate this problem.

Heredity and iron balance in women

The present finding that the magnitude of the menstrual blood loss is genetically controlled is consistent with the individual constancy of the menstrual blood loss reported previously (Hallberg and Nilsson, 1964b Hallberg, Högdahl, Nilsson, and Rybo, 1966b) and the agreement in mean menstrual blood loss at different ages (Hallberg, Högdahl, Nilsson, and Rybo 1966a)

The iron balance is determined by the absorption of iron and the loss of iron from the body There are no studies showing that the variation in absorption of iron in normal women is influenced by heredity The familial incidence of iron deficiency anaemia in women was explained by the hypothesis of Lundholm (1939, 1959) that there is a genetic component in the iron absorption variation. The hereditary influence on the iron balance in women, however may be explained by the present finding of a genetic control of the menstrual loss of iron.

The critical iron balance situation in women, reflected by the high incidence of iron deficiency anaemia, and the observation that there is a close relationship between the menstrual blood loss and iron deficiency imply that the observed genetic control of the variation in menstrual blood loss is of great importance for the iron balance Disregarding other factors affecting iron balance the present finding of a genetic control of the main variable in iron loss from the female body may also imply that differences in the incidence of iron deficiency in different populations may have a genetic basis.

SUMMARY

The menstrual blood loss was measured twice in 18 monozygotic and 24 dizygotic twin pairs. The intrapair variance was statistically lower in the monozygotic than in the dizygotic twins indicating a genetic influence on the menstrual blood loss.

Parity as an environmental factor

Parity was studied as an environmental factor The reason for this was that parity is of special interest because of the hormonal and anatomical changes which occur during pregnancy Furthermore in clinical practice information is sometimes obtained from patients that the menstrual blood loss has changed after childbirth.

In order to examine the effect of parity the series was subdivided into the equal-parity group and the unequal-parity group. A comparison between these groups shows that both for MZ and DZ twins the mean intrapair differences and the intrapair variances are higher and the intraclass correlation coefficients lower in the unequal parity group than in the equal-parity group The difference between the V_{mz} values in the two groups was not statistically significant The difference between the V_{dz} values however was statistically significant ($F=2\ 78$ df 8 and 16 $p<0\ 05$) These findings indicate that a difference in parity is of importance as an environmental factor and may also indicate that in MZ twins the stronger hereditary component of variation counteracts the effect of this environmental factor The strong hereditary influence on menstrual blood loss is obvious in the equal parity as well as in the unequal parity group through the statistically significant V_{dz} V_{mz} ratio.

The subdivision of the material into nulliparous and nulliparous-parous groups was made in an attempt to investigate the influence of parity as such and not only of a difference in parity These groups are very small, but a comparison shows the same tendency as when the equal-parity and the unequal-parity groups were compared. The difference between the V_{dz} values was statistically significant ($F=3\ 72$ df 7 and 11 $p<0\ 05$) It must be emphasized however that the subjects in these groups constitute a major proportion of the subjects in the equal-parity and the unequal-parity groups.

On the basis of the comparisons between the various subgroups, it seems reasonable to conclude that parity may influence the menstrual blood loss. However the present study does not give sufficient information on the relation between

APPENDIX

Data for the Monozygotic Twins

•	Age	Parity	Blood Loss, First and Second Period (ml)	Intraperiod Difference (ml)	Mean Value of the Periods (ml)	Intrapair Difference (ml)	Comments
13	9		20 0– 18 6	1 4	4 3	9 5	
			24 0–23 6	4	3 8		
43	20		35 8–29	6 8	32.4	3 8	
			3 26	5	8.6		
55	20		58 5–60 8	2.3	59 7	3 4	
			46 2–46 4		46 3		
70			26 7–26 8	0	26 7	3 7	
			24 8–35 9		30 4		
20			3 7–27 3	3 8	25.5	3 4	
			9 2–24 9	5 7	22		
4	23		3 8–24 5	8.7	20.2	4	
			17 3–20	9	18 8		
4	23		8 0– 9 4	4	8 7	0.4	
			16 20 5	4 4	8 3		
41	36		32 4–3		3 8	17 7	
			8	8	14		
42	37		8–22 9		22.4	5	
			27 9–27	5	27 6		
51	29	3	26 9–60	33 3	43 8	3	
		3	46 7–46 5		46 6		
	22		24 3– 5 4	8 9	9 9	8.6	
			30 0–27	3	8 5		
34	29		0– 8 7	3 3	4	9	
			4 9		3 5		
24	29		20 8 8	3	4 5		
			5 8– 4	6 6	5		
	29		4 3– 4 3		4 3		
			9 7– 4 5	4 8			
67	38	3	27 3–33	5 7	30	5	Concordant bloc
			3 3–26 7	3 4	5		groups
33	36		34 4–38 8	4 4	36 6	3 3	
			38 8.4	9 7	33 3		
62	37	3	42 4–4 8	6	42	6	
			34 8–37 3	5	35		
46	32		2– 3 8	5	6	3 5	Concordant bk
		3	6 5 26 7	34 8	44		groups

In an attempt to evaluate the effect of parity as an environmental factor certain subgroups of the series were studied. Thus a comparison was made between one group in which the members of a twin pair were equal with respect to parity and another group in which the twins within one pair were of unequal parity. In the latter group there was a higher intrapair variance and a lower intraclass correlation coefficient in both MZ and DZ twins. The same tendency was observed when a group in which both twins of a pair were nulliparous was compared with another group consisting of pairs in which one twin had born children the cotwin had not.

The results of the study allow the conclusion that menstrual blood loss is influenced by heredity and by parity as one environmental factor

Acknowledgements

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The statistical analysis was designed by fil. mag Håkan Lindström

No.	Age	Parity	Blood Loss, First and Second Period (ml)	Interperiod Difference (ml)	Mean Value of the Periods (ml)	Intrapair Difference (ml)	Comments
				9	22.8	31.5	
53	38		22 3–23	8	54 3		
			45 3–63 3	7.1	30.1	8 0	
27	2		33 6–26 5	2.1	22		
			1.0–23.1			48	
17	23		67.6–75	7 5	71.4		
			33 6–12.7	20 9	23.2		
					15.3	6.4	
5	26		4 8– 5.7	9	21 7		
			22.1 2 .2	9		.0	
8	3		5 3– 6.5	.2	5 9		
			38 6–35	3.4	36 9		
			30 9–40.	9 6	35 3	12.1	
	34		20 7–25 6	4 9	23.2		
					24 1	5 9	
5	37		24 7–23 5	.8	30		
			30 9–29		6.7	54	
50	37		14 2– 9	5	70 7		
			78 6–62 7	5 9	42	1.2	
36	34		39 5–44 4	4 9	40 8		
		5	35 –46 4	3		7.4	Excluded from the statistical analysis
37	27		4	–	6 6		
			9 5 3 6	5 9		7.4	
40	28		6 22 6	6 5	9 4		

No.	Age	Parity	Blood Loss, First and Second Period (ml)	Intraperiod Difference (ml)	Mean Value of the Periods (ml)	Intrapair Difference (ml)	Comments
13	22	0	27.2	–	–	2.0	Excluded from the
		0	29.2	–	–		statistical analysis
45	24	0	5.6	–	–	7.4	
		0	13 0	–	–		
3	22	1	17 2–11 5	5 7	14.4	16 6	
		0	31 0	–	–		
35	26	1	24.6– 8 5	16 1	16 6	7 7	
		2	24 3	–	–		

Data for the Dizygotic Twins

No.	Age	Parity	Blood Loss, First and Second Period (ml)	Intraperiod Difference (ml)	Mean Value of the Periods (ml)	Intrapair Difference (ml)	Comments
71	19	0	19.4 23 5	4 1	21 5	0.6	
		0	21.9–22.3	0.4	22.1		
66	19	0	32.6–34 5	1.9	33.6	0.6	
		0	34.1–34 3	0.2	34.2		
64	19	0	60.0–23.0	37.0	41 5	9.5	Disconcordant
		0	48.9–53.1	4.2	51 0		blood groups
54	20	0	15.5–12.6	2.9	14.1	21.2	
		0	34 6–35.9	1 3	35.3		
30	23	0	27 5–22.6	4 9	25.1	14.8	
		0	9 9–10.7	0.8	10.3		
9	23	0	14.5–20.6	6 1	17 6	2.5	
		0	22.6–17 6	5.0	20.1		
1	23	0	44.1–71 1	27 0	57 6	16 6	
		0	40.6–41 3	0 7	4 0		
58	25	0	35.3–30.4	4.9	32.9	10 0	
		0	42.7–43 1	0.4	42 9		
20	26	0	25.4 23 0	2 4	24 2	1 3	
		0	23 8–22.0	1.8	22.9		
19	26	0	10.1–14.7	4.4	6.9	9 2	
		0	6.8– 8 5	1 7	7 7		
25	31	0	57.2 48 7	8 5	53 0	36 0	
		0	12.2–21 8	9.6	17 0		
68	37	1	16 1–23.6	7 5	9.9	6.	
		1	12.2–15.3	3.1	13 8		
48	38	1	38.4–53 9	15 5	46	25 9	
		1	16 3–24 2	7 9	20.3		
44	27	2	13.4 14.7	1 3	4 1	8 6	
		2	9 7– 1 6	8.1	5 5		
28	29	2	24.3–22.0	2.3	23.2	0.1	
		2	24.2–21 9	2.3	23.1		

Rybo, Göran, Acta obst. et gynec. scandinav. 45, 4 1966

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PLASMINOGEN ACTIVATORS IN THE ENDOMETRIUM

I. Methodological aspects

BY

GÖRAN RYBO

The fibrinolytic activity of the human endometrium has been studied by many authors during the last fifty years (Halban and Frankl 1910 Whitehouse 1914 Kross 1924 Huggin, Vail and Davies, 1943 Smith and Smith, 1945 Philips Butler and Taylor 1956 Page Glendening, and Parkinson, 1951 Kullander and Källén, 1961 Fuhrmann, 1962 Todd, 1962 Rybo 1964) However the methods used in these studies differed considerably and this makes it difficult to compare the results Furthermore, many of the determinations of the fibrinolytic activity in endometrial tissue were probably influenced by fibrinolytic components present in residual blood in the tissue samples.

Astrup (for a general survey see Astrup 1956) has shown that the fibrin splitting enzyme plasmin, or fibrinolysin, is produced by transformation of a precursor in the blood, plasminogen or profibrinolysin. This transformation is effected by various activators present in blood, urine and tissues (Astrup and Permin 1947 Lewis and Ferguson, 1950 Astrup 1956 Todd 1958 Stamm 1962) The clinical significance of the tissue activators is not entirely clear A hypothetical consideration is that these enzymes by activating plasminogen into plasmin, may influence the haemostatic mechanism (Astrup, 1956) The over-all fibrinolytic activity in the endometrium is of course dependent on factors other than the concentration of plasminogen activators Thus, inhibitors against fibrinolytic enzymes localized in blood

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50 and 100 mg, was superficially cleaned from blood and homogenized with 3 ml distilled water in a Potter homogenizer To determine the nitrogen content 0.5 ml was removed. Solid KSCN was added until a final concentration of 2 *M* was obtained. The sample was shaken for one hour and centrifuged for 20 minutes at 7000 r.p.m. The supernatant was removed and the residue was additionally extracted twice with 2.5 ml 2 M KSCN The three supernatants were combined and diluted with 7 parts of distilled water and acidified to pH 1.0 with 1 *N* HCl whereby the acid stable tissue activators were precipitated. After centrifugation, the sediment was redissolved in 10 ml 2 *M* KSCN Solid NaHCO was added for neutralization and the dilutions 3 4 1 2 1 4, 1 8 were made with 2 *M* KSCN

DETERMINATION OF THE ACTIVITY ON FIBRIN PLATES

The plate method for determination of the fibrinolytic activity was originally described by Astrup and Müllertz (1952) and has been modified by Nilsson, Sjoerdsma, and Waldenstrom (1960) In the present work, a further modification was made by adding agar to the plate (Holmström, 1965) The advantage of such an arrangement is that the consistency of the fibrin layer becomes firm, allowing wells to be cut. Thus the variable extension of a drop put on the surface is avoided, with the result that a circular lysis zone is obtained which can be measured with a high degree of accuracy

30 ml 1 per cent bovine fibrinogen solution and 3 ml thrombin solution (Topostasin, Roche) containing 100 N.I.H. units (National Institute of Health) per ml were added to a glass bottle containing 270 ml tris buffer (pH = 7 8, $\mu = 0.15$) with 2 per cent agar (Noble Agar Difco) During this procedure the bottle was stored in a water bath at 40 C The contents of the bottle were immediately distributed on 10 plexiglass plates where it was allowed to coagulate Each plate contained 28 ml. Five wells with

Kindly supplied by AB Kabi Stockholm.

"Olofsson plate (diameter 70 mm) from Koagulationslaboratoriet Medicinska kliniken Allmänna sjukhuset, Malmö

or tissue may be of importance as well as other fibrinolytic com ponents of the tissue However the plasminogen activators can be determined separately but quantitative methods for deter mination of inhibitors are not available at the present time.

The plasminogen activators seem to be firmly bound to protein (Permin, 1947 Astrup and Stage 1952) and specific sol vents are needed to extract them. Astrup and Stage (1952) found that the activators could be obtained in a soluble form after treatment with KSCN

On the basis of this finding, Astrup and Albrechtsen (1957) devised a method for quantitative determination of tissue activators of plasminogen. This method permits separation of in-hibitors In addition the labile blood activators which otherwise would interfere with the measurements are destroyed.

The method has been applied to various tissues of different species Plasminogen activators have been found in most organs (Albrechtsen, 1959) However the variations between dif ferent species and different individuals were great.

The present study deals with the methodological errors in volved when the plasminogen activators are determined by the above-mentioned method. In another investigation (Rybo 1966) the concentration of these enzymes in the endometrium is studied in a series of women suffering from menorrhagia In order to evaluate the results of this latter study an understanding of the methodological errors is necessary

MATERIAL

The material consisted of pig heart tissue obtained as soon as possible after the animal was killed and of endometrial tissue obtained by means of curettage

The tissue was frozen immediately at −20 C and stored at this temperature until used.

METHODS

EXTRACTION PROCEDURE

In principle the extraction was performed according to Astrup and Albrechtsen (1957) A piece of tissue usually between

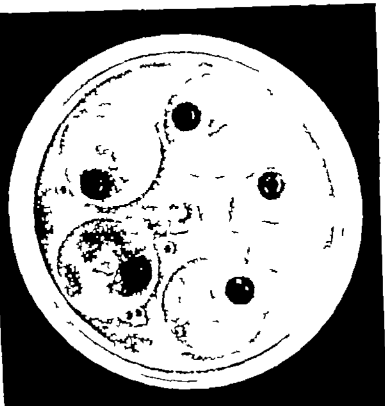


Fig. A fibrin plate with lysed zones. At the centres of the zones are the wells in which the sample to be investigated was deposited.

same standard preparation was used in all the experiments in this study.

CALCULATIONS AND STATISTICAL METHODS

One standard unit was defined as the activity obtained on a fibrin plate (average of 5 determinations) with 0.05 ml of the undiluted standard solution.

Among various models examined, the relationship between the activity concentration and the lysed area was best expressed by the following equation

a diameter of 5 mm were made on each plate and 0 05 ml of the sample was deposited into each well. One plate (5 determinations) was used for every dilution level. The plates were incubat ed at 37 C for 18 hours. The lysed zones were measured by means of a microscope (magnitude 6 3 ×) with a hair scale in one of the ocular lenses Two diameters, perpendicular to each other were measured and the product of these was taken as the surface area and expressed in mm (Astrup and Müllertz, 1952) Thus the zone measured is the area of the circumscribed square The mean value of 5 determinations on each plate was calculated. Figure 1 shows a fibrin plate with the five lysed zones.

By heating the fibrin plates at 85 C for 35 minutes, the plasminogen is destroyed. This arrangement thus permits separate determination of the plasmin activity (Lassen, 1952)

DETERMINATION OF NITROGEN IN THE TISSUES

Nitrogen was used as reference substance and thus the activity can be expressed as standard units per mg nitrogen.

The nitrogen content of the tissues was determined according to Fels and Veatch (1959) and Strid (1961) 0.05 ml tissue homogenate in distilled water was digested with 0 1 ml of the combustion acid used in the Kjeldahl procedure. The digest was mixed with 3 ml 4 *N* sodium acetate buffer (pH 5.5) and diluted with 7 ml water Samples were taken (volume 2 ml) for the ninhydrin reaction. $(NH_4)_2SO_4$ was used as a standard For each tissue sample, two nitrogen determinations were made. The method is suitable for determinations of amounts as low as 10 μg nitrogen.

THE STANDARD PREPARATION

An extract of fresh pig heart tissue prepared as described above was used as a standard However the extraction was carried out only once. The extract was distributed into small glass bottles and lyophilized. The contents of one bottle (100 mg) were used for one experiment only The powder was dissolved in 75 ml 2 *M* KSCN and the activity was determined in serial dilutions The

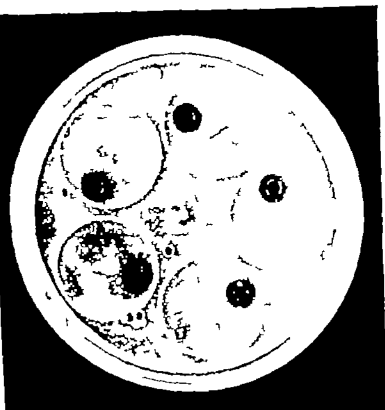


Fig A fibrin plate with lysed zones At the centres of the zones are the wells in which the sample to be investigated was deposited

same standard preparation was used in all the experiments in this study

CALCULATIONS AND STATISTICAL METHODS

One standard unit was defined as the acti ity obtained on a fibrin plate (a erage of 5 determinations) with 0.05 ml of the undiluted standard solution.

Among various models examined, the relationship between the activity concentration and the lysed area was best expressed by the following equation

$$\log A = b + k \cdot \log c \qquad (1)$$

where A is the activity measured as the area of the lysed zone expressed in mm c is the concentration b and k are constants. This equation indicates that the relation between log A and log c is linear The same relation was found by Astrup and Albrechtsen (1957)

The constants b and k which determine the regression function were estimated on the basis of the logarithmic values according to the least square method, and the correlation coefficient was calculated.

If no qualitative differences exist between the standard and the sample the functions related to concentration and activity will be linear and parallel in a suitable transformed graph (Finney 1952) Thus if the slopes of the curves for the standard and the sample are similar qualitative differences may be excluded This was tested according to the method for linear regression. If $p < 0.001$ a significant difference was considered to exist and the sample was excluded. If the slopes did not differ significantly the coefficients of regression were coordinated to get the most reliable determination of the slopes

If these criteria of linearity and parallelism were fulfilled, the activity of the sample was calculated as follows W mg tissue is homogenized in 3 ml of distilled water and 0.5 ml is removed for nitrogen determination. The nitrogen content is found to be N μg in 0.05 ml of the homogenate. The plasminogen activators are thus extracted from $W - \frac{W}{6}$ mg tissue and finally dissolved in 10 ml KSCN of which 0.05 ml is taken for determination of the activity on a fibrin plate. S standard units are calculated from the standard curve Thus the activity of the sample is

$$\frac{S \times 10 \times 6}{5\,W \times 0.05} \text{ standard units/mg wet weight} \qquad (2)$$

But in each mg wet weight there is

$$\frac{N \times 60}{W \times 1000} \text{ mg nitrogen} \qquad (3)$$

Thus the activity is

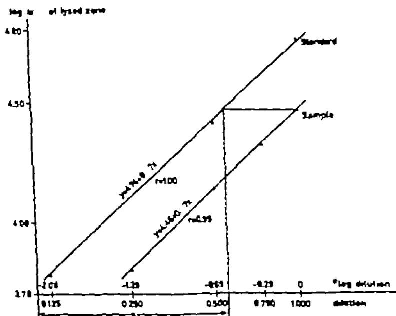


Fig An example of calculation of the activator activity in an endometrial sample The function lines and correlation coefficient are calculated for tandard and sample. The parallelism is evident. S represents the activity of the undiluted sample expressed in standard units calculated from the standard curve (for further explanation see text).

$$\frac{S \times 60 \times W \times 1000}{N \times 60 \times 5\,W \times 0.05} \text{ standard units/mg nitrogen, or} \qquad (4)$$

$$\frac{4000\,S}{N} \text{ standard units/mg nitrogen} \qquad (5)$$

Figure 2 illustrates an example of the calculations.

The mean value standard deviation, standard error of mean, Student s t, the regression and correlation coefficients were all calculated with conventional methods (Brownlee 1961) The index of precision λ was estimated according to Gaddum (1933) as the ratio between the residual standard deviation and the slope of the regression line The precision index L (Gaddum

1953) is the reciprocal of λ and was also used as a criterion of precision.

RESULTS

A. CRITERIA OF VALIDITY

The validity of the assay procedure used in the present study as has been pointed out before is dependent on the function between concentration and activity being linear in a double logarithmic graph and the dilution curves of the standard and of the sample being parallel. These criteria of validity were examined.

I. *Linearity*

The linear relationship between the logarithmic values of the lysed zones and the concentrations of the plasminogen activator was not mathematically tested. However the graphical recording indicates that the relation is best expressed as a linear function (Fig. 2). Furthermore the correlation coefficients give certain information on the deviation of the values observed from a straight line. In 115 samples or standard solutions in a clinical series, (Rybo 1966) the correlation coefficient was equal to or above 0.9 in all except 5 samples. In four of these the correlation coefficients were above 0.8 and in the fifth above 0.7.

II. *Parallelism*

The parallelism between the dilution curves of the standard and of the sample was established in each experiment by means of a t test. In the clinical series no statistically significant difference was obtained in 73 out of 82 endometrial samples analysed. In 9 samples however the slope was significantly different from the slope of the standard curve. Thus a qualitative difference between these samples and the standard could not be ruled out and consequently they had to be excluded from the series. No special clinical findings were observed in the patients from whom these excluded samples originated.

B CRITERIA OF RELIABILITY

Borth (1952) proposed four reliability criteria for a biological assay precision, sensitivity accuracy and specificity The method used in the present work to determine plasminogen activators in tissue was examined according to three of these criteria. The criterion of specificity was not analysed in this investigation, but this has been done by Astrup and Albrechtsen (1957) No lysis occurred when 2 M KSCN alone was applied to the fibrin plates

I. *Precision*

The precision of the method used to determine tissue activators of plasminogen was analysed in different ways

The precision is first given in terms of standard deviation and coefficient of variation calculated from repeated experiments on pig heart tissue. This approach also gives an estimate of the error involved in each step of the procedure as well as the over all error of the method. Furthermore, the results obtained in the clinical series were used to calculate the indices of precision λ and L.

1 *The stability of the standard preparation and the error of the activity determination on the fibrin plate*

To compare results from different experiments, the standard preparation used must be stable The standard activities from 51 consecutive experiments performed during more than one year are presented in Table I. Two different fibrinogen batches were used during this time. The mean values and the standard deviations of the activities in each dilution are given. The standard deviation is an estimate of the variation between different standard solutions, but includes also the variation caused by the error introduced by the preparation of the plates

From Table I it also appears that fibrinogen bath Qdx 15 ga e a less sensitive plate than fibrinogen batch Qdx 14 which means that the dilution 1 8 gave lysis only in three experiments when the former fibrinogen was used.

1953) is the reciprocal of λ and was also used as a criterion of precision.

RESULTS

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Table III. The Error Inherent in the Extraction Procedure and the Plate Determination.

Dilution	Number of Determinations	Mean Value of the Lysed Zones in mm²	Standard Deviation
1	10	84	8.7
3:4	10	74	5.5
1:2	10	65	4.7
1:4	10	50	6.2

activators were extracted from each portion and the activities were determined on fibrin plates. The results are given in Table III. The variation includes the error of the activity determination on the plates as well as the error involved in the extraction procedure. The coefficient of variation was 10 per cent for the undiluted sample.

3. *The error of the nitrogen determination*

The nitrogen content of 10 equal portions of the same homogenate of pig heart tissue was determined. The mean value was 36.1 μg in 0.05 ml and the standard deviation 2.6 μg, corresponding to a variation coefficient of about 7 per cent.

4. *The error of the calculation of the slopes. The over-all error of the method*

An evaluation of the error introduced by calculation of the slope of the dilution curve was obtained in the following way. The areas of the lysed zones recorded from the experiment described in Section 2 were plotted in the usual manner in a double logarithmic graph. The slopes of these 10 samples were calculated. The mean slope and its standard deviation are given in Table IV. The variation coefficient was 16 per cent.

The over-all error of the method was then evaluated from the above mentioned 10 samples. A standard curve was plotted, and the parallelism between the curve of the samples and of the standard was established by means of a t test. The slopes of the

Table I *The Variation in the Activity of the Standard Preparation.*

Dilution	Fibrinogen Qdx 4			Fibrinogen Qdx 5		
	Number of Determinations	Mean Value of the Lysed Zones in mm	Standard Deviation	Number of Determinations	Mean Value of the Lysed Zones in mm²	Standard Deviation
1 1	31	110	9 11	20	90	6.08
1 2	31	87	7 55	20	69	6.00
1 4	31	65	7 37	20	51	4.40
1 8	25	48	5 98	3	43	-

Table II. *The Variation in a Single Activity Determination of the Standard Preparation on Fibrin Plates (Plate Error)*

Dilution	Number of Determinations	Mean Value of the Lysed Zones in mm	Standard Deviation
1 1	9	78	4.0
3 4	8	63	1 6
1 2	9	54	4.9
1 4	8	41	3 3

The coefficient of variation for the undiluted standard solution was 7 per cent when fibrinogen batch Qdx 15 was used and 8 per cent when the plates were prepared from fibrinogen batch Qdx 14

In one experiment, one set of plates prepared on the same occasion was used to evaluate the error of one plate preparation only The activity of a standard solution (in serial dilution) was estimated on 35 plates (each gave a mean value of 5 determina tions) The results are shown in Table II The coefficient of variation is 5 per cent for the undiluted solution.

2 *The error of the extraction procedure*

To evaluate the error of the extraction procedure the following experiment was performed. 750 mg pig heart tissue was homog enized in 2 *M* KSCN and divided into 10 equal portions The

Table III. *The Error Inherent in the Extraction Procedure and the Plate Determination.*

Dilution	Number of Determinations	Mean Value of the Lysed Zones in mm	Standard Deviation
1		84	8.7
3 4		74	5.5
2	0	65	4.7
4	0	50	6.2

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Table IV *The Variation in the Method Including Extraction Procedure Plate Determination and Determination of the Slopes of the Dilution Curves*

Number of Assay	Mean Value of the Slopes	Standard Deviation	Mean Value of the Activity in Standard Units/mg N	Standard Deviation
10	0 379	0.0601	103	21.9

sample and the standard were coordinated as described before. The nitrogen content of each of the 10 samples was determined. The activity value of each sample was calculated from the standard curve and expressed as standard units per mg nitrogen. The mean activity and standard deviation were calculated. The results are given in Table IV The variation coefficient amounted to 21 per cent. This estimate of the methodological error includes the error involved in every step of the procedure and is thus an expression of the over all error of the method.

The estimate of the precision described above is based on experiments performed on pig heart tissue. It is not reasonable to assume that the precision of the method is changed when applied to human endometrium but it is desirable to scrutinize the procedure even during these conditions

Such an evaluation of the precision was made by using the results obtained in the clinical series The indices λ and L were calculated. These indices are convenient estimates of the precision since they are independent of the experimental design and the units used and they also permit a comparison between the precision of the methods used in various laboratories.

The indices λ and L were determined in 72 assays in the clinical series The mean value and standard error of the mean for λ was 0.118 ± 0.0047 and for L 9.13 ± 0.307 However these values were calculated from the natural logarithms of the measured values. To obtain the λ and L values corresponding to $^{10}\log$ of the measured values the value of λ had to be multiplied and the value of L divided by a constant which is 0.4343 This transformation gives a mean value of $\lambda = 0.051 \pm 0.0020$ and of $L = 21.02 \pm 0.707$

Table V *The "Recovery" of the Method.*

Wet Weight	Calculated Activity Area of Lysed Zones in mm²	Estimated Activity Area of Lysed Zones in mm²	Recovery = Estimated Activity in Per Cent of Calculated
100	84	79	94
75	75	73	97
62.5		69	–
50	64	65	101
37.5	58	52	90
			Mean recovery 96

II. *Sensitivity*

A rough estimate of this characteristic may be obtained by testing the lowest concentration of a urokinase solution which gave a distinct lysed zone. This was found with a urokinase solution (Löven) containing 0.5 units per ml.

III. *Accuracy*

The accuracy of a method is usually determined by recovery ex-periments implying addition to a sample of a known amount of the substance to be determined. Obviously this was not possible in the present investigation. However an approximate estimate of the accuracy was obtained in the following way. Pig heart tissue (325 mg) was homogenized in 2 M KSCN and divided into 5 portions containing 100, 75, 62.5, 50 and 37.5 mg. The activators were extracted and the activity of serial dilutions was determined on fibrin plates. The parallelism between the five dilution curves was established. The portion containing 62.5 mg tissue was considered as "standard" when the activity of the other four samples was calculated. The relation between the estimated and calculated activity gave a concept of the "recovery" of the method. The results are given in Table V. The average "recovery" was 96 per cent.

C. Activity estimation on heated fibrin plates

In some cases KSCN-extracts from endometrium and pig heart

Table IV *The Variation in the Method Including Extraction Procedure Plate Determination and Determination of the Slopes of the Dilution Curves*

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C ACTIVITY ESTIMATION ON HEATED FIBRIN PLATES

In some cases KSCN-extracts from endometrium and pig heart

tissue were examined on heated fibrin plates for evidence of fibrinolytic activity No lysis or only very small zones were obtained implying that the fibrinolytic activity obtained on non-heated plates is mainly due to plasminogen activators and not to plasmin

DISCUSSION

The fibrinolytic activity of the endometrium has been estimated mostly by means of the effect on various substrates of either tissue fragments (Halban and Frankl, 1910 Ishizuka, Kawashima Kano Terao and Takeuchi, 1964) or of homogenates in saline or water (Huggins Vail, and Davies 1943 Page Glendening, and Parkinson 1951 Fuhrmann, 1962) However these procedures are not quantitative (Albrechtsen, 1958) and furthermore information is usually lacking as to whether the activity is due to proteolytic enzymes such as e g plasmin or activators of plasminogen.

On the basis of the finding that tissue activators of plasminogen could be converted to a soluble form with KSCN (Astrup and Stage, 1952) a method for quantitative determination of tissue activators was devised by Astrup and Albrechtsen (1957) The advantages of this method are that the determinations are not affected by the presence of labile blood activators which are destroyed by acidification (Astrup and Sterndorff 1956) and that inhibitors against fibrinolytic enzymes can be separated.

In the present study this method was examined with special reference to the criteria of validity and reliability To calculate the results mathematical and statistical methods were applied which require fulfillment of certain validity criteria Thus a linear relationship between the concentration of activators and the area of the lysed zones must exist in a double logarithmic graph. Furthermore it must be proved that the dilution curves of the standard preparation and the sample are parallel in a double logarithmic graph otherwise qualitative differences between the two solutions may exist.

The criterion of linearity seemed to be valid However the linearity was not mathematically calculated. Evidence obtained

from graphical recording and from the correlation coefficient calculated for each sample and standard preparation strongly supported the assumption that there was a linear relationship. The criterion of linearity has previously been pointed out by Astrup and Albrechtsen (1957)

The criterion of parallelism between the standard and the sample is required for many enzymatic determinations when the activity is estimated from a standard curve (Finney 1952) The validity of such a parallelism in connection with determination of plasminogen activators was emphasized by Astrup and Albrechtsen (1957) However their evaluation of parallelism was performed graphically Sometimes, difficulties may arise when evaluating the parallelism from a graphical recording only This was the reason for applying statistical methods in the present study when testing whether there was any significant difference between the slopes of the sample and the standard.

The stability of the standard preparation must be reliable when comparisons are made between various assays. The standard preparation used in the present investigation showed a high degree of stability A great advantage was that the same pig heart preparation could be used as the standard in all experiments throughout the study

The precision of the method was estimated in each step of the procedure and expressed in terms of standard deviation and variation coefficient. The error of a single activity determination on a fibrin plate (average of 5 determinations) amounted to 5 per cent Astrup and Müllertz (1952) reported an error of 11 per cent for a single plasmin determination and 6 per cent when trypsin was determined. Even if these figures are not comparable to the error obtained when determining plasminogen activators it is evident that the error of a single activator determination on fibrin plates was low Partly this may be due to the adding of agar to the plates. The agar-containing fibrin plate was firm, allowing circular wells to be made Thus the variable extension of a drop of a test solution deposited on the fibrin surface was avoided. The lysed zones became circular and could be measured with a high degree of accuracy The largest error of the method was introduced when the slope of the curve was estimat

ed. This is in agreement with the findings of Astrup and Albrechtsen (1957)

The precision of the method was further evaluated on the basis of the results obtained in a series of patients. This approach gave a concept of the precision of the method when applied to endometrial tissue. Thus the precision was calculated in terms of λ and L (Gaddum 1953) The figures obtained for these indices indicate a high degree of precision since $\lambda < 0.2$ or $L > 5$ implies a precise assay in routine works (Loraine 1958) These indices of precision have not been applied previously to the method used in the present study

Obviously it was not possible to evaluate the accuracy of the method correctly as a critical estimate of the accuracy involves recovery experiments by adding known amounts of the substance to be determined to the sample. However an approximate evaluation of the recovery was obtained by extracting the plasminogen activators from different amounts of tissue. The sample containing the median amount of tissue was chosen as standard, and the recovery was calculated as estimated activity in per cent of calculated activity However it must be emphasized that this approach to recovery estimation is not a correct evaluation of the accuracy

SUMMARY

The method for determining tissue activators of plasminogen originally described by Astrup and Albrechtsen (1957) was examined with reference to validity and reliability The method involves repeated extraction of enzymes with KSCN and determination of the fibrinolytic activity in serial dilutions on fibrin plates The activities recorded were plotted against the concentrations in a double logarithmic graph the slopes of the dilution curves of the standard and the sample were estimated mathematically Finally the activity value was calculated as standard units per mg nitrogen. The lyophilized standard preparation was made from pig heart tissue and showed a high degree of stability The criterion of linear relationship between the enzyme concentration and the area of the lysed zone in a double

logarithmic graph was established as was the criterion of parallelism between the standard and the sample.

The precision of the method was evaluated in each step of the procedure. The largest error was introduced when determining the slope of the dilution curve. The over-all error amounted to 21 per cent. The precision indices λ and L_r were estimated in 72 assays The mean value of λ was 0.051 ± 0.0020 and of L 21.02 ± 0.707 indicating a high degree of precision.

A concept of the sensitivity and accuracy was obtained.

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PLASMINOGEN ACTIVATORS IN THE ENDOMETRIUM

II. Clinical Aspects

V riation in the concentration of plasminogen ctivators during the menstrual cycle and its relation to menstrual blood loss

BY

GÖRAN RYBO

Menorrhagia is a common disorder (Hallberg, Högdahl, Nilsson, and Rybo 1966) and it is a well known clinical ex perience that in a large number of patients with this condition, ordinary gynæcological examinations do not reveal any pathological findings in the uterus. On account of the suggestion by Astrup (1956) that tissue activators of plasminogen may cause an increased local fibrinolysis which may interfere with hæmostatic mechanisms Albrechtsen (1956 a) pointed out that the concentration of plasminogen activators in the endometrium might be of importance for the genesis of abnormal menstrual bleeding. In some conditions often associated with heavy blood loss from the uterus such as endometrial hyperplasia and proliferative bleeding, a high concentration of plasminogen activators in the endometrium has also been described (Albrechtsen, 1956 a Ishizuka, Kawashima, Kano Terao, and Takeuchi, 1964 Todd, 1964)

However very little information is given in the literature on the fibrinolytic activity of the endometrium of women with menorrhagia, menorrhagia being defined as heavy menstrual blood loss from a normal-sized uterus with a histologically normal endometrium. The possibility that an increase in local fibrinolysis in the uterus may cause menorrhagia was pointed out by Nilsson and Björkman (1965) However Todd (1964) found no increased fibrinolytic activity in the endometrium of 16 women

regarded as suffering from menorrhagia in comparison with "normal women In Fuhrmann's series (1962) two cases of menorrhagia are included. In these subjects no increased fibrinolytic activity was observed in endometrial homogenates in saline. The methods used by Fuhrmann (1962) and Todd (1964) are, however not specific for tissue activators of plasminogen.

Usually it is difficult to evaluate the magnitude of the menstrual blood loss on the basis of anamnestic information only (Rybo 1964) In the above-mentioned papers by Fuhrmann (1962) and Todd (1964) no objective data is given on the menstrual blood loss To establish the diagnosis of menorrhagia, suitable methods for quantitative determination of the menstrual blood loss must be applied. Furthermore, the upper limit of the normal blood loss must be defined By using a method devised by Hallberg and Nilsson (1964) Hallberg, Högdahl, Nilsson, and Rybo (1966) found the upper limit of the normal menstrual blood loss to be in the range 60–80 ml, and concluded that a blood loss above 80 ml should be regarded as menorrhagia.

Another difficulty arises when studying the relation between plasminogen activators in the endometrium and the menstrual blood loss The fibrinolytic activity in the endometrium varies during the menstrual cycle and is higher in the secretory than in the proliferative phase (Caffier 1930 Fuhrmann 1962 Todd 1962) Furthermore there is evidence showing that the activity in the secretory phase increases as the cycle progresses reaching a maximum just before menstruation (Page Glendening, and Parkinson 1951 Philips Butler and Taylor 1956 Albrechtsen 1956 a) However there have been no systematic studies to clarify more precisely the variation in the concentration of plasminogen activators during the secretory phase. If such a variation exists the cycle day of biopsy must be standardized when different subjects are compared

The aim of the present investigation was

1 to study the variation of tissue activators of plasminogen in the endometrium during the menstrual cycle with special reference to the premenstrual phase.

2 to study the concentration of plasminogen activators in the

endometrium in a group of women with menorrhagia and in a group of women with normal menstrual blood loss

MATERIAL

Endometrial tissue was obtained on *the first day of the period* from 34 subjects divided into the following groups

Group I Twelve subjects with a normal-sized uterus and with an average menstrual blood loss below 80 ml.

These women were admitted to the hospital either because of subjective complaints of heavy blood loss (9 subjects) but in whom the measured blood loss was below 80 ml, or because of infertility (3 subjects) The examinations of the infertile women did not reveal any gynaecological abnormalities Hysterosalpingography was performed on five subjects in the group. No fibroids in the uterine cavity were found. There were no clinical reasons why hysterosalpingography should be performed in the other subjects. In one subject, biopsy in the proliferative phase of the cycle was also performed.

Group II Twenty-two subjects with a normal-sized uterus and with an average menstrual blood loss above 80 ml. These women were admitted to the hospital on account of menorrhagia and/or iron deficiency In all subjects except three (CO BO and MY in Table IV) hysterosalpingography was performed, and the presence of fibroids in the uterine cavity was excluded Seven women in this group were also subjected to biopsy during the premenstrual phase

In 44 samples from 39 women, the plasminogen activator concentration was determined *before* the onset of the period. Ten of these subjects had uterine fibroids The number of samples examined on the various days in the menstrual cycle is given in Table I

In 10 of the women in the whole series the plasminogen activator concentration was determined on different days in two cycles For each patient in the series data with respect to age, parity and special observations made is given in Tables III and IV

Table I *Number of Women and Biopsies in the Series*

Group	Number of Women	Number of Biopsies Performed on Different Days prior to the Period						Total Biopsies
		Proliferative Phase	Day 4	Day 3	Day	Day	Day o[a]	
Women with menstrual blood loss below 80 ml	17	2	2	–	1	1	12	18
Women with menstrual blood loss above 80 ml	48	5	6[b]	5	13	9	22	60
Total	65	7	8	5	14	10	34	-8

=first day of the period.

[b] in one woman, the biopsy was performed on the 6-th day before the period.

Table II. *Mean Values and Standard Error of Means of the Plasminogen Activator Activity in Standard Units/mg N on the First Day of the Period and on Different Days in the Premenstrual Phase The Figures in Parentheses Indicate Number of Assays Performed*

Group	Day before the Period					
	Proliferative Phase	4	3			o[a]
Women with menstrual blood loss below 80 ml						
Activity	94 (2)	54 (2)	–	30 (1)	96 (1)	42 ± 15.6 ( )
Women with menstrual blood loss above 80 ml						
Activity	48 ± 7 5 (5)	32 ± 4 7 (5)	55 ± 15.2 (5)	89 ± 14.0 (3)	86 ± 31.0 (9)	331 ± 60 (22)
Total series						
Activity	6 ± 14.7 (7)	38 ± 5 5 (7)	55 ± 5.2 (5)	85 ± 13 7 (14)	177 ± 29.2 ( )	264 ± 42.0 (34)

=first day of the period.

The following criteria had to be fulfilled in all subjects in the series.

1 The endometrium should be of normal histological appearance corresponding to the day of the cycle on which the biopsy was performed.

2 The dilution curves of the standard and the sample should be parallel when plotted in a double logarithmic graph (for further information see Rybo 1966)

Nine samples had to be excluded because the dilution curves were not parallel with the dilution curve of the standard. No particular findings to explain the deviations were observed in these patients.

3. The activity must be recorded in serial dilutions of the endometrial extract. Usually the activity was recorded in three to five dilutions. In two endometrial samples obtained on the first day of the period, activity was recorded only in undiluted extracts. Thus it was impossible to establish the linearity of the dilution curves and the samples were excluded. However exceptions from this criterion were made in 5 cases. In 3 of these samples were obtained four days before the period in one three days before the period while one was obtained during the proliferative phase of the cycle During these days, the activity is usually very low The samples were included in the material although they did not fulfill the validity criteria, and may be regarded only as semiquantitative data. It should be emphasized, however that the activity values of these samples were not used in any calculation of the relation between activator activity and menstrual blood loss The values were only used to evaluate the variation in the activity during the premenstrual phase.

Out of all endometrial samples examined throughout the study no activity could be registered in 7 samples. These are not included in the material described above. Three of the samples were obtained between 3 and 10 days before the calculated period. During these days the activity is low Two samples originated from patients subjected to biopsy two days before the period. One of these patients died of uremia two months later In the other as well as in one endometrium obtained one day before the period, no reason can be given why any activity could

not be recorded. Technical errors cannot be ruled out. Finally one endometrium without activity was obtained on the first day of the period However the wet weight of this sample was extremely low 10 mg, and the activity of the extract was probably too low to be detected

METHODS

One part of the endometrial tissue obtained was subjected to histological examination at the Department of Pathology The remainder was immediately frozen at −20 C and stored at this temperature until used.

The day of the cycle selected for biopsy was calculated on the basis of the last period and the menstrual interval. If the biopsy was to be performed on the first day of the period the patient was instructed to come to the clinic as soon as possible but not later than 24 hours after observation of blood flow

Biopsies were usually performed without anæsthesia. When anæsthesia was required a barbiturate was used as a basal anæsthetic. Supplementary anæsthesia with oxygen and nitrous oxide was given.

A satisfactory agreement between the calculated day of the cycle and the histological appearance was established by means of microscopy of the endometrium. Furthermore, when biopsy was performed before the first day of the period the patient was instructed to observe carefully the onset of menstruation.

The methods of determining plasminogen activators and nitrogen in the tissue have been described earlier (Rybo 1966) When the fibrinolytic activity in a homogenate of physiological saline was determined, 0 1 ml saline was used for each mg tissue

The method used to determinate menstrual blood loss was that of Hallberg and Nilsson (1964 a) The hæmoglobin of venous blood was determined as cyanmethæmoglobin. The average of two menstrual periods was taken as an estimation of the menstrual blood loss Thus in most subjects the menstrual blood loss was measured during one cycle before and one cycle after the one in which the biopsy was performed. In 5 subjects in Group I and 2 subjects in Group II only single measurements were carried out.

Table III. *Data and Results Obtained for Women with an Average Menstrual Blood Loss below 80 ml*

Patient	Age	Parity	Biopsy on Day before Period	Plasminogen Activator Activity in Standard Units/mg N	Activity of Homogenates in Saline in Lysed Area in mm	Mean Blood Loss in ml	Comments
LW	2	II	0	1	84	76	One measurement of menstrual blood loss
LL	24	I		83	80	2	One measurement of menstrual blood loss
VA	24	O		190	84	64	One measurement of menstrual blood loss
MO	26	I		4	[illegible]	69	
IF	28	O		74	78	3	
EE	28	O		222	83	30	One measurement of menstrual blood loss
HR	3	I	0	324	37	74	
GG	37	II	0	68		65	
EK	40	I		146	2	34	
GH	4	II		72	[illegible]	62	
			Prol. phase	44	62		
MJ	42	III		44	27	66	
MB	43	III		66	74	25	One measurement of menstrual blood loss
UG	8	II	1	96		4	One measurement of menstrual blood loss
HE	30	I	2	30	53	80	
LU	35	III	4	6		72	Subserous fibroids of the uterus found at laparotomy
IJ	4	I	4	46	48	30	Uterus bicornuted
MH	32	O	Prol phase	44	94	54	

Conventional methods were applied for the calculation of standard error of mean, correlation coefficient, Student's t test, and the Wilcoxon two sample rank test (Brownlee 1961).

not be recorded. Technical errors cannot be ruled out Finally one endometrium without activity was obtained on the first day of the period However the wet weight of this sample was extremely low 10 mg, and the activity of the extract was probably too low to be detected.

METHODS

One part of the endometrial tissue obtained was subjected to histological examination at the Department of Pathology The remainder was immediately frozen at –20 C and stored at this temperature until used

The day of the cycle selected for biopsy was calculated on the basis of the last period and the menstrual interval If the biopsy was to be performed on the first day of the period the patient was instructed to come to the clinic as soon as possible but not later than 24 hours after observation of blood flow

Biopsies were usually performed without anæsthesia. When anæsthesia was required, a barbiturate was used as a basal anæsthetic. Supplementary anæsthesia with oxygen and nitrous oxide was given.

A satisfactory agreement between the calculated day of the cycle and the histological appearance was established by means of microscopy of the endometrium. Furthermore when biopsy was performed before the first day of the period, the patient was instructed to observe carefully the onset of menstruation

The methods of determining plasminogen activators and nitrogen in the tissue have been described earlier (Rybo 1966) When the fibrinolytic activity in a homogenate of physiological saline was determined 0 1 ml saline was used for each mg tissue.

The method used to determinate menstrual blood loss was that of Hallberg and Nilsson (1964 a) The hæmoglobin of venous blood was determined as cyanmethæmoglobin. The average of two menstrual periods was taken as an estimation of the menstrual blood loss Thus in most subjects the menstrual blood loss was measured during one cycle before and one cycle after the one in which the biopsy was performed. In 5 subjects in Group I and 2 subjects in Group II only single measurements were carried out.

Patient	Age	Parity	Biopsy on Day before Period	Plasminogen Activator Activity in Standard Units/mg N	Activity of Plasminogen in Saline as Lysed Area in mm²	Mean Blood Loss in ml	Comments
RE	43	III		100	–	1 5	Uterine fibroids
MA	44	II	1	88		232	
GM	44	I		296	–	89	
HS	49	I		60	64	141	One measurement of menstrual blood loss
KP	32	O		4		95	Uterus bicornis? Fibroids?
SB	34	O	2	4	49	20	
MS	34	III	2	[illegible]		85	
IP	36	I		7	67	313	
				8			
EK	41	O	2	79	42	127	Uterine fibroids
			3	31			
MN	41	I	2	20		52	Uterine fibroids
RW	42	I	2	50	55	291	Uterine fibroids
MM	45	III		178	–	04	Uterus slightly enlarged
AF	49	III	2	46	68	92	Uterus slightly enlarged
			2	137	–		
GK	9	O	3	8	64	77	
BO		O	3	06		02	
IF	48	IV	3	58		98	Uterine fibroids
EK	8	O	4	32		122	Activity in undiluted extract only
			4	9	18		
HH	4	O	4	5		75	Activity in undiluted extract only
MS	4	V	4	35	62	258	Activity in undiluted extract only
GJ	46	II	4	47		00	Uterine fibroids
			6	45	54		
MB	4	O	Prol. phase	36		96	One measurement of menstrual blood loss
KM	35	I	Prol phase	43		258	Uterine fibroids Activity in undiluted extract only
MJ	4	III	Prol. phase	43		225	
EH	46	II	Prol phase	78		06	Uterine fibroids

In this patient, another determination of the plasminogen activator activity in the endometrium was made four months later. Her average blood loss was then 71 ml. The activity values obtained were [illegible]

Table IV *Data and Results Obtained for Women with an Average Menstrual Blood Loss above 80 ml*

Patient	Age	Parity	Biopsy on Day before Period	Plasminogen Activator Activity in Standard Units/mg N	Activity of Homogenates in Saline as Lysed Are in mm²	Mean Blood Loss in ml	Comments
CO	16	O	0	149	88	93	
BO	18	O	0	6	77	90	One measurement of menstrual blood loss
UW	19	O	0	683	55	81	No passage through the tubes at x-ray examination
			3	62	–		Activity in undiluted extract only
MK	21	O	0	128	–	344	
EL	27	I	0	313	–	127	One measurement of menstrual blood loss—thereafter pregnant
BF	30	I	0	277	130	216	
MC	31	II	0	175	99	220	
IL	36	III	0	79	60	168	
			Prol. phase	42	56		
IJ	37	IV	0	564	52	181	
AO	37	II	0	184	104	132	
			1	167	–		
GT	37	IV	0	413	75	150	
			1	266	–		
IH	38	IV		127	71	188	Uterus slightly enlarged. Cavity normal at x ray examination
IF	40	IV	0			125	
GS	41	I	0	259	64	401	
MS	41	I	0	466	55	152	
			1	328	109		
MY	41	III	0	136	116	92	
IL	41	II	0	3	40	162	
IJ	41	III	0	154	96	368	Ovarian endometriosis
			2	12	47		
MK	44	III	0	266	96	304	
ML	45	II	0	419	96	129	
GG	46	II	0	1132	104	232	
EK	47	IV	0	948	112	204	

Patient	Age	Parity	Biopsy on Day before Period	Plasminogen Activator Activity in Standard Units/mg N	Activity of Homogenates in Saline on Lysed Area in mm²	Mean Blood Loss in ml	Comments
KE	43	III	1	00	–	115	Uterine fibroids
MA	44	II	1	188	–	232	
GM	44	I		296	–	89	
HS	49	I		60	64	141	One measurement of menstrual blood loss
KP	32	O		41		95	Uterus bicornis? Fibroids?
SB	34	O		4	49	120	
MS	34	III		19	–	85	
IP	36	I		71	67	1 3	
				8			
EK	4	O		79	42	27	Uterine fibroids
			3	3	–		
MN	41	I	2	190	–	52	Uterine fibroids
RW	42	I		50	55	29	Uterine fibroids
MM	45	III		178	–	104	Uterus slightly enlarged
AF	48	III		44	68	92	Uterus slightly enlarged
				37			
GK	9	O	3	8	84	177	
BO	2	O	3	06		02	
IF	48	IV	3	58		98	Uterine fibroids
EK	28	O	4	32		222	Activity in undiluted extract only
			4	19	38		
IH	4	O	4	25		175	Activity in undiluted extract only
MS	4	V	4	35	62	258	Activity in undiluted extract only
GJ	46	II	4	47		100	Uterine fibroids
			6	46	54		
MB	24	O	Prol. phase	36		96	One measurement of menstrual blood loss
KM	35	I	Prol. phase	43		258	Uterine fibroids Activity in undiluted extract only
MJ	4	III	Prol. phase	43		225	
EH	46	II	Prol. phase	78		06	Uterine fibroids

In this patient, another determination of the plasminogen activator activity in the endometrium was made four months later. Her average blood loss was then 71 ml. The activity values obtained were 84 standard units/mg N and 70 mm

Table IV *Data and Results Obtained for Women with an Average Menstrual Blood Loss above 80 ml*

Patient	Age	Parity	Biopsy on Day before Period	Plasminogen Activator Activity in Standard Units/mg N	Activity of Homogenates in Saline as Lysed Area in mm²	Mean Blood Loss in ml	Comments
CO	16	O	0	149	68	93	
BO	18	O	0	6	77	90	One measurement of menstrual blood loss
UW	19	O	0	683	55	81	No passage through the tubes at x-ray examination
			3	62	–		Activity in undiluted extract only
MK	1	O	0	1 8	–	344	
EL	27	I	0	313	–	127	One measurement of menstrual blood loss —thereafter pregnant
BF	30	I	0	277	130	216	
MC	31	II	0	175	99	220	
IL	36	III	0	79	60	168	
			Prol. phase	42	56		
IJ	37	IV	0	564	52	181	
AO	37	II	0	184	04	132	
			1	167	–		
GT	37	IV	0	413	75	150	
			1	266	–		
IH	38	IV	0	127	71	188	Uterus slightly enlarged. Cavity normal at x-ray examination
IF	40	IV	0	212	11	125	
GS	41	I	0	259	64	401	
MS	41	I	0	466	55	152	
			1	328	109		
MY	41	III	0	136	1 6	92	
JL	41	II	0	3	40	162	
IJ	41	III	0	154	96	368	Ovarian endometriosis
			2	1	47		
MK	44	III	0	266	96	304	
ML	45	II	0	4 9	98	29	
GG	46	II	0	1 32	104	32	
EK	47	IV	0	948	11	204	
			2	148	–		

The results shown in Figs. 1 and 2 include all subjects in the series independent of the magnitude of the menstrual blood loss However the same tendency is observed within each group when the series was divided into those with menstrual blood loss be low and above 80 ml, even if only a few subjects with a men strual blood loss below 80 ml were studied during the premenstrual days (Table II)

In 10 subjects the activities were determined on different days in two cycles. The results are presented in Fig 3 and Tables III and IV The premenstrual increase of the concentration of tissue activators also is evident from this result.

II. *Plasminogen activators in the endometrium determined on the first day of the period in relation to the menstrual blood loss*

The results are presented in Fig. 4 and in Table II In both groups there was a great variation between the individuals. In Group I (12 subjects) the mean value of the activities was 142 ± 15.6 standard units per mg nitrogen and in Group II (22 subjects) 331 ± 60 1 standard units per mg nitrogen. The dif

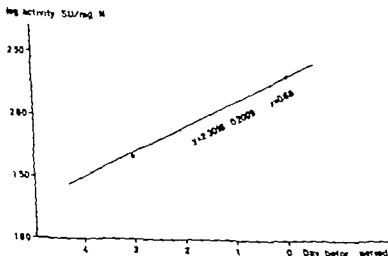


Fig The relation between the plasminogen activator activity and time before the period Each point indicates the mean activity value obtained on the various days in the premenstrual phase

RESULTS

The results obtained for each subject in the series are presented in Tables III and IV

I *Plasminogen activators in the endometrium during the menstrual cycle*

The concentration of plasminogen activators was investigated on the first day of the period, on each of the four days before the period, and in the proliferative phase. The results are shown in Table II and are illustrated in Figs 1 and 2

The concentration of activators gradually increased during the premenstrual days and reached a maximum on the first day of the period The increase of the concentration of activators during the premenstrual days is statistically significant. In a semilogarithmic graph the relation between time before the period and the activity can be expressed by the equation given in Fig 2

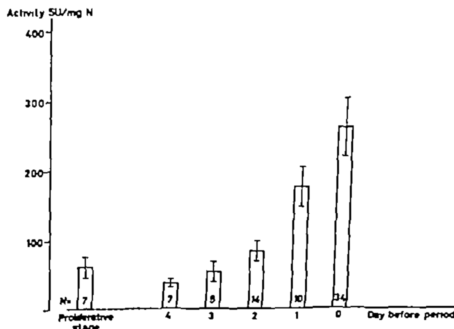


Fig 1 The mean values of the plasminogen activator activity in the endometrium during the proliferative and the premenstrual secretory phase and on the first day of the period. The standard errors of the means are indicated.

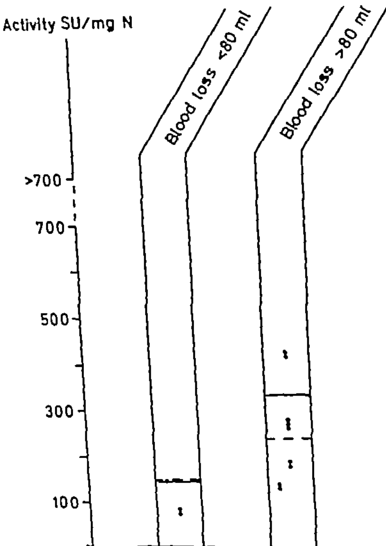


Fig. 4. The plasminogen activator activity in the endometrium on the first day of the period in subjects with menstrual blood loss below and above 80 ml. Each point represents one subject. The solid horizontal lines indicate the mean values and the dotted lines the median values of each group

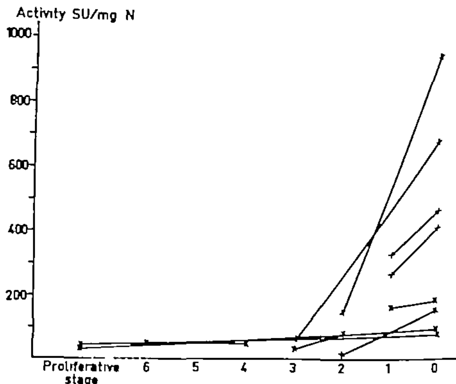


Fig 3. The fibrinolytic activity in the endometrium on different days of the menstrual cycle in 10 subjects Each line represents one individual. The activities were determined on different days in two cycles

ference between these mean values was statistically significant when tested with Student s t test ($t = -2.285$ $df = 32$, $p < 0.05$) However the use of this test is not justified as the variance estimates of the two groups are statistically different. Therefore the Wilcoxon two sample rank test was applied. It was found that *the distribution of the values was statistically different in* the two groups i e the median value of Group I (138 standard units per mg nitrogen) was statistically lower than the median value of Group II (225 standard units per mg nitrogen) ($z = -2.03$ $p < 0.02$) The correlation coefficient between the magnitude of the menstrual blood loss and the concentration of plasminogen activators was also calculated. However this coefficient was not statistically different from zero

It should be noted that the mean activity obtained on the first

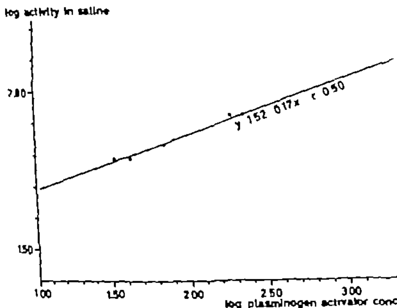


Fig. 5. The relation between the plasminogen activator activity and the fibrinolytic activity of the endometrium estimated in homogenates of saline.

blood has also been shown by many authors (for references see Albrechtsen 1956 b) Furthermore, Albrechtsen (1956 a and b) found that menstrual blood has a high concentration of plasminogen activators of the same type as in the human endometrium. The fibrinolytic activity of endometrial tissue has also been shown by various investigators (for references see Rybo 1966) Therefore many factors support the assumption that local fibrinolysis occurs in the uterus mainly due to the abundance of plasminogen activators in the endometrium.

The possibility that a high concentration of this enzyme is an etiological factor for abnormal menstrual bleeding was pointed out by Albrechtsen (1956 a) and Nilsson and Björkman (1965) However there has been no previous systematic study of the clinical significance of a high concentration of plasminogen activators in the endometrium of women with menorrhagia.

The present method to determine plasminogen activators in tissue was originally described by Astrup and Albrechtsen

day of the period in Group I was lower than that obtained one day before the period in Group II. This indicate that the premenstrual increase of the activator concentration is greater in women with menorrhagia than in women with a normal blood loss

III *The fibrinolytic activity of endometrial homogenates in saline*

Besides the concentration of plasminogen activators the fibrinolytic activity was determined in homogenates prepared in physiological saline in 19 endometrial samples obtained before the period and in 31 samples obtained on the first day of the period The mean activity value for the samples obtained before the onset of the period, expressed as the area of the lysed zone on a fibrin plate was 58±5 2 mm The mean value for the samples from the first day of the period was 88±4 8 mm The difference is statistically significant ($t=4.098$ $df=48$ $p<0.001$)

The distribution of the 31 samples obtained on the first day of the period in the two clinical groups outlined above was as fol lows Group I 11 and Group II 20 samples The mean value for Group I was 83±6 9 mm and for Group II 90±6.4 mm The difference is not statistically significant. The activity value for each woman is given in Tables III and IV

Studies were also made to see if there was any correlation between the plasminogen activator concentration and the activity obtained in saline homogenates Such a correlation was found, as is shown in Fig. 5.

IV *Other analyses*

Tests were made to find out if there was any correlation be tween the concentration of plasminogen activators and the age or the parity of the women, the nitrogen content or the wet weight of the endometrium. No such correlations were found.

DISCUSSION

The observation that menstrual blood does not clot spontaneously owing to absence of fibrinogen (Whitehouse 1914 Smith and Smith, 1945) has led to the assumption that local fibrin olysis occurs in the uterus The fibrinolytic power of menstrual

but that their fragility became progressively more marked in the secretory phase. In conditions associated with necrosis, the lysosomes rupture and their enzymes are released into the cells.

The lysosomal theory may be in accordance with the premenstrual increase of the plasminogen activators in the endometrium. Thus, sexual steroids may induce a release of lysosomal enzymes accompanied by necrosis and onset of bleeding. The fibrinolytic enzymes may take part in the regenerative process by dissolving fibrin deposits. Furthermore, blood clots formed in the uterine cavity are lysed. However, further studies are needed to get more information on these aspects of the onset of the menstrual bleeding.

The results of the present study clearly show the necessity of measuring the enzyme concentration on the same day in the menstrual cycle when various subjects are compared. The first day of the period was chosen for the following reasons. This day of the cycle is easy for the patient to recognize, it can be verified histologically by the presence of desquamation and the enzyme concentration is high on this day. Furthermore, if the activator concentration affects the menstrual blood loss, it seems reasonable to suggest that it is the enzyme concentration present at the onset of bleeding which is of importance.

For practical reasons the biopsy had to be performed at a convenient time during the first 24 hours of the menstrual blood flow. Therefore, no consideration could be taken of any possible variation of the activator concentration occurring during these 24 hours. Furthermore, scanty bleeding may occur from the endometrium before real menstruation begins, which would mean that a lower activity would be recorded than if the biopsy had been performed later. Certainly, these circumstances may influence the activity determinations and may contribute to the great individual variation in the series. However, no systematic difference was observed between the various groups in the series with regard to the time of biopsy during the first day of the period or to the occurrence of scanty premenstrual bleeding.

In the present study, the menstrual blood loss was determined as the mean value of two measurements, one before and one after the biopsy. The values obtained in such a way are fairly

(1957) The errors of the method have been analysed in a previous paper (*Rybo 1966*)

Two important factors require special attention when a comparison is made between the activator concentration in the endometrium in different subjects with different menstrual blood losses The first is the variation of the activator concentration within the menstrual cycle and the second is that the diagnosis of menorrhagia can be established in a reliable way

The results of the present study show that there is a successive increase in the activity during the premenstrual days. Such an increase took place irrespective of the magnitude of the menstrual blood loss Twelve of the subjects examined during the premenstrual phase had uterine fibroids. However the endometria of these subjects were all histologically normal and corresponded to the day of the cycle on which biopsy was made. Therefore it was justified to include these samples to demonstrate the premenstrual variation in the plasminogen activator activity

The results obtained are in principle in agreement with those published by Albrechtsen (1956a) and with other authors who state that the fibrinolytic activity of the endometrium (measured in different ways) successively increases during the menstrual cycle (Page Glendening, and Parkinson 1951 Philips Butler and Taylor 1956 Fuhrmann 1962 Todd 1962)

Astrup (1956) assumed that tissue activators of plasminogen are released in necrotic or damaged tissue Thereby plasminogen in blood will be converted into plasmin. This enzyme is considered to be of importance for the regeneration of damaged tissue through its proteolytic effect on fibrin deposits

Lach and Ali (1964) showed that fibrinolytic enzymes in tissues are found in the lysosomal fraction of the cell According to de Duve (for a general survey see Ciba Foundation symposium 1963) the lysosomal enzymes are released by different agents and it was shown by Sheib (1963) that sexual steroids may affect this release Furthermore Bitensky (1963) found that, during the proliferative phase the lysosomes of the glandular epithelium of the endometrium were comparatively stable

the normal group. The heavy blood loss in these 11 subjects cannot be explained by a high concentration of plasminogen activators in the endometrium. There were no abnormalities with respect to bleeding or coagulation time, the number of blood platelets, the differential leucocyte counts or the content of fibrinogen in blood. Further studies are needed to find out the reason for menorrhagia in these women.

Little information is given in the literature on the fibrinolytic activity of the endometrium in women with menorrhagia. Furthermore, the menstrual blood loss has not been objectively evaluated in any of the studies made and exact information is lacking on which day of the cycle the biopsy was made. Todd (1964) did not find any difference in fibrinolytic activity between menorrhagic and normal" women. However the method used differed considerably from the one used in the present study and is not specific for tissue activators of plasminogen.

The results obtained in the present study are in principle in agreement with the findings of Flähmig, Sieg, and Vogel (1965) who found a higher fibrinolytic activity of the menstrual blood from patients with menorrhagia than in menstrual blood from normal subjects. However their series of women with menorrhagia was very heterogenous and no measurements of the blood loss were performed.

No regression function was found to exist between the magnitude of the menstrual blood loss and the concentration of plasminogen activators. However many factors make such a correlation unlikely. As mentioned before the measurements of the blood losses were performed during other periods than the one in which the activity was determined. An individual variation between periods in either the blood loss or the activity will influence the correlation. Furthermore in many enzymatic systems, there is a balance between active enzymes and inhibitors. The presence of inhibitors in the fibrinolytic system is generally accepted (Astrup, 1956). Probably the magnitude of the menstrual blood loss depends on the relation between activators and inhibitors localized in blood or tissue. Consequently heavy blood loss may be an effect of a relatively high concentration of activators or a relatively lack of inhibitors. However this hypothesis

good estimates of the blood loss of normal women as the in dividual variation from one menstruation to another is small (Hallberg and Nilsson, 1964 b) The variation in the blood loss in women with menorrhagia is greater (Nilsson and Rybo unpublished) and, occasionally the menstrual blood loss may be below 80 ml However the patients with menorrhagia in this study were followed with consecutive measurements during sev eral menstrual periods. The average value of all measurements exceeded 80 ml for each patient. Thus the diagnosis of menor rhagia would not have changed, if the average value of several measurements had been used.

The use of the mean value of two measurements made it also possible to avoid the influence of variations in menstrual blood loss which may occur in the course of several months. Further more when the enzyme activity was related to the magnitude of the menstrual blood loss it seemed most convenient to use the blood loss measured during the periods near the one during which the biopsy was performed. The blood loss during the period of investigation could not be related to the activity because the biopsy procedure usually reduced the blood loss.

In one patient (ML in Table IV) the plasminogen activator activity was determined on the first day in two different periods. The time interval between these two determinations was four months. At the second determination a much lower activity value was obtained than at the first determination. However the woman's menstrual blood loss was also lower during the periods near the second determination Of course it is impossible to draw any conclusions from the result from one single case. It was not possible to determine the activity on the first day of two different periods in other subjects. Thus the information is in sufficient to decide whether there is a variation in the activator concentration between different cycles and whether an individual variation in the menstrual blood loss is related to a variation in the activator concentration.

The concentration of plasminogen activators in women with menorrhagia on the first day of the period was higher than in women with normal blood loss. However among the 22 subjects in Group II eleven had activity values within the same range as

SUMMARY

The fibrinolytic activity of the endometrium, determined as tissue activators of plasminogen, has been investigated on different days during the premenstrual phase and in the proliferative phase. The concentration of plasminogen activators in the endometrium increased successively before the onset of menstruation and reached the highest value on the first day of the period.

In a group of women with menorrhagia there was a statistically significant increase in the proportion of subjects with a high concentration of plasminogen activators in the endometrium on the first day of the period as compared with a control group with normal menstrual blood loss.

The fibrinolytic activity in the endometrium was also determined in tissue homogenates of saline. The activity was statistically higher on the first day of the period than during the premenstrual days. For women with menorrhagia, the activity in saline homogenates was slightly higher than for the control group but the difference was not statistically significant. There was a linear relationship in a double logarithmic graph between the plasminogen activator concentration and the fibrinolytic activity in saline homogenates.

On the basis of the results obtained, the conclusion is drawn that, in some cases of menorrhagia, a high concentration of plasminogen activators in the endometrium may be of aetiological importance

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cannot be experimentally proved or rejected as methods for quantitative determination of inhibitors are not available. The presence of inhibitors may of course, also contribute to the lack of a regression between plasminogen activators and the magnitude of menstrual blood loss

The fibrinolytic activity in the endometrium was also determined in tissue homogenates in saline. This approach however gives only incomplete information on the fibrinolytic activity in the tissue since blood activators and inhibitors may influence the determinations (Astrup and Albrechtsen, 1957) Furthermore, the determination is not quantitative. After extraction with saline, still more activity can be yielded by another extraction with KSCN (Albrechtsen, 1958) In the present study a relation was found between the activator activity and the fibrinolytic activity in saline homogenates This relationship can be expressed as a linear function in a double logarithmic graph which corresponds to an exponential function, implying that the activity in saline did not increase to the same extent as the plasminogen activator activity This may depend on various factors such as the presence of inhibitors and blood activators in the saline homogenate and the various milieus obtained in KSCN and NaCl.

The fibrinolytic activity in saline was statistically higher on the first day of the period than on the preceeding days The activity in saline was also higher for women with menorrhagia than for the control group but the difference was not statistically significant. The results obtained are in agreement with the results from the plasminogen activator study

On the basis of this discussion and the results obtained it seems logical to conclude that a high concentration of plasminogen activators in the endometrium may be of aetiological importance in some women with menorrhagia The underlying mechanism cannot be fully explained but it is assumed that the activators by activating plasminogen, cause transformation into plasmin. This enzyme gives rise to fibrinolysis which may affect the haemostatic mechanisms

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Aus der Frauenklinik (Professor Sam Brody) der Universität in Göteborg, Schweden

TUBARRESEKTIONEN BEIM AFFEN MIT UND OHNE APPLIKATION VON GROSSEN INTRAPERITONEALEN DOSEN GLUKOKORTIKOID

VON

KURT SWOLIN

Greenhill hat 1937 das Ergebnis einer grossen internationalen Rundfrage publiziert, in dem die Erfolgsrate von Tubarplastiken mit 4,4 % lebend geborener Kinder angegeben wird. Seit dieser Zeit ist es jedem Gynäkologen mehr oder weniger geläufig, dass die Resultate von Eileiteroperationen ähnlich unbefriedigend oder noch schlechter sind. In Schweden hat Westman (1951) an Hand eines grossen eigenen Materials die Konzeptionsrate mit 6,4 % angegeben. Vereinzelte Verfasser mit grosser persönlicher Erfahrung haben bessere Resultate publiziert (Meaker 1935 Holtz, 1951 Comninos 1954 Chalier 1956 Murray 1956 Vara, 1959 Green Armytage, 1960 Palmer 1960 Shirodkar 1961)

In Schweden rechnet man mit 15 % unfreiwillig sterilen Ehen (Westman, 1951) Ähnliche Ziffern gibt es von anderen Ländern, besonders in der westlichen Hemisphäre. Eine recht neue Publikation (Dtsch. med. Wschr 1960) aus der Bundesrepublik Deutschland besagt dass etwa 20 % der Ehen kinderlos sind. Grob gerechnet ist der Anteil der Frau an der sterilen Ehe etwa 50 % (Vara 1959) Der sogenannte Tubarfaktor ist von grosser Bedeutung fur die Sterilität der Frau. Sein Anteil an der Unfruchtbarkeit der Frau ist mit etwa 50 % sicherlich recht gut eingeschätzt. (Schultze 1939 Westman, 1944 Winson, 1946 Pollosson 1948 Rubin, 1951 Bernhard, 1955 Gallucci und Mueller 1956 Pobedinski, 1956 Vara, 1959

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er wieviel Zeit braucht er zur Restitution? Wieviel muss man nähen, benötigt man eine exakte und sehr dicht stehende Naht oder benötigt man vielleicht überhaupt keine Naht?

Gleichzeitig soll dieser mit einer Eileiteroperation am Menschen vergleichbare Modellversuch dazu dienen, die intraperitoneale Applikation von grossen Dosen Glukokortikoid zu studieren. Verhindern oder vermindern Steroide am Schluss der Operation lokal appliziert, intraperitoneale Adhäsionen? Welchen Einfluss hat die Glukokortikoidgabe auf die Resektionsstelle? Kann man es wagen, eine der von Thomaschek (1959) als optimal für die Ratte angesehene Dosierung beim Affen und somit auch mit aller Wahrscheinlichkeit beim Menschen anzuwenden? Thomascheks optimale Dosis von 10 mg Hydrocortison pro Ratte bedeutet eine Menge von etwa 3000 mg für eine Frau von 60 kg Gewicht.

Die Bindegewebshemmung durch Glukokortikoide

Schon 1934 berichtet Kosdoba über die Verzögerung der Wundheilung durch eine vermehrte Nebennierenfunktion. Nach seinen Beobachtungen spielt das Gefäßsystem hierbei eine wichtige Rolle er beobachtet ein Zurückbleiben der Gefässneubildung. 1940 sieht Menkin eine Hemmung der durch Exsudat oder durch „Leukotaxin hervorgerufenen Kapillarpermeabilität in Gegenwart von Nebennierenextrakten.

Nach der Synthese des Cortison folgt bald die Einführung dieses Präparates in die klinische Forschung (Hench *et al.* 1949) und damit öffnen sich viele Gebiete der Medizin für die Forschung mit diesem neuen, faszinierenden Präparat. Im gleichen Jahr beobachten Ragan und Mitarbeiter (1949) als Nebenwirkung des ACTH eine Retardierung der Wundheilung. Im folgenden Jahr ziehen Ragan und Mitarbeiter (1950) aufgrund von experimentellen und klinischen Beobachtungen den Schluss, dass Cortison das Bindegewebe hindert, auf ein Trauma zu antworten. Meier und Mitarbeiter (1952) stellen fest dass Cortison das Wachstum von Fibroblastenkulturen hindert (Huhn und andere Tiere) nachdem bereits vorher in sehr eindrucksvollen Versuchen mit Hilfe des Wattegranuloms an der Ratte (1950) ge-

Green Armytage 1960 Cox, 1964 Gonzales 1964 Steptoe 1965) Zu beachten ist hierbei jedoch dass die von allen Verfassern mitgeteilten Zahlen mit aller Wahrscheinlichkeit Minimalziffern sind da die Coelioskopie fast nie in die Untersuchungen mit einbezogen worden ist

Aus dem oben Gesagten geht eindeutig hervor dass ein großer Teil der sterilen Ehen – die ja dank ihres Anteils von 15–20 % aller eingegangenen Ehen eigentlich ein volksgesundheitliches Problem sind – durch die mangelnde Transportfähigkeit der *Eileiter hervorgerufen wird. Diese wird zum grossten Teil durch* Verwachsungen und Verklebungen hervorgerufen (Westman, 1944) Die Behandlung dieser ist im grossen und ganzen rein operativ (Roberts 1965) trotz der erstaunlichen, aber leider nicht reproduzierbaren guten Resultate einiger Autoren mit der Hydropertubation von unwegsamen Tuben (de Moraes und Peano 1958) Die Resultate von Eileiterplastiken sind aber wie bereits erwähnt auch heute immer noch entmutigend (Bergman 1963 van Coeverden de Groot, 1963 Sager 1964 Roberts 1965) Nicht zuletzt ist es vor allem die an und für sich natürliche und in einem anderen Zusammenhang durchaus *sinnvolle Eigenschaft der Natur nach einem intraperitonealen* Trauma Verwachsungen zu bilden, die allzuoft primär – das heisst intraoperativ – gute Resultate zunichte macht.

Unsere Kenntnisse uber das postoperative Verhalten der Tuben sind gering (Gepfert 1939 Castallo 1950) Einmal ist es die relative Geringfugigkeit des Eingriffes bei einer Tubarplastik, die verhindert dass es kurz nach einer Operation wegen einer Komplikation zu einer Reoperation kommt die ihrerseits gestattet, das postoperative Verhalten der Tuben zu studieren. Zum anderen ist es der Mangel an geeigneten Versuchstieren da nur Primaten einen dem Menschen ähnlichen Bau und Form der Eileiter haben.

Fragestellung

Die folgenden Versuche sind einfache Grundversuche zur Frage des Verhaltens der Tuba Fallopii nach operativer Querresektion. Wie verhält sich der Eileiter nach einer Querresektion wie heilt

Leider ist dieser Schutzmechanismus nicht immer erwünscht oder nützlich und führt durch einen Überschuss an Bindegewebsbildung zu unerwünschten Folgen in Form von Narbengewebe und Adhäsionen. Die Fähigkeit der Serosa, zu verlöten, macht einer seits erst die Bauchchirurgie möglich, andererseits sind aber seit jeher die postoperativen intraabdominalen Verwachsungen für die Chirurgen eine ständige Plage gewesen und haben manchen guten Operationserfolg vereitelt. In ganz besonderem Masse gilt dies natürlich auch für Fertilitätsoperationen und Tubarplastiken (Solomons 1935 Gepfert, 1939 Westman 1944 Rutherford *et al* 1949 ten Berge und Tik Lok, 1954 Comninos, 1954 Rock, 1957 Siegler und Hellmann, 1963 Caplier 1964 Swolinzky 1965 Palmer 1966)

Bereits vor der Jahrhundertwende (Wegner 1877 v Dembowski, 1888 Graser 1888 Stern, 1889 Walthard, 1893 Martin, 1895 ten Brink, 1898) haben sich Chirurgen und Gynäkologen um die Adhäsionsprophylaxe bemüht. Fast unzählige Arbeiten sind mit diesem Problem verknüpft, wie man in etwa an der Zahl der folgend zitierten Autoren mit sehr umfangreichen Literaturübersichten zur Frage der postoperativen Adhäsionen sehen kann (Graser 1888 Flesch Thebesius 1920 Löhnberg, 1922 Vogel 1923 Kubota, 1924 Wereschinski, 1925 Ladwig, 1928 Clairmont und Meyer 1929 Turunen, 1933 Boys 1942 S Krook 1947 Myburgh, 1953 Fries 1956 Thomaschek, 1959 Connolly 1960 Laurentaci und Oliva, 1961 Kern und Kuhbier 1964)

Die Entdeckung des Cortison und seiner bindegewebshemmenden und antiinflammatorischen Eigenschaften hat natürlich sofort dazu geführt, dass auch dieses Mittel versucht wurde um Adhäsionen zu vermeiden oder zu vermindern. Scheinberg und Saltzstein (1951) sehen nach parenteraler Gabe von Cortison eine beträchtliche Reduktion der intraabdominalen Adhäsionen bei sowohl Ratten wie Hunden. Zu ähnlichen Resultaten kommen Odell und Mitarbeiter (1951) bei ihren Versuchen mit Ratten und intramuskulärer Applikation von Cortison. Ekeström (1952) sieht gute Resultate in seinen Versuchen an Ratten und beschreibt hierbei erstmals auch eine intraperitoneale Applikation des Cortison. Hubay und Mitarbeiter (1953) be

zeigt worden war, dass Cortison imstande ist, die Bindegewebsneubildung kräftig zu reduzieren.

In den 50er Jahren erscheinen dann zahlreiche Arbeiten, die alle eindeutig die antiinflammatorische Wirkung oder – für die Zwecke dieser Arbeit besser ausgedrückt – die Hemmung der Reaktion des Bindegewebes durch Glukokortikoide zeigen. Da es den Rahmen dieser Arbeit überschreiten würde, auf alle Arbeiten näher einzugehen, sollen im folgenden nur einige in aller Kürze zitiert werden. Spain *et al.* (1950): Spärsame Fibroblastenproliferation nach Cortison, Maus. Alrich *et al.* (1951): *Cortison verzögert die Bildung aller reparativen Zellelemente des Mesenchyms*, Albinoratte. Cornman (1951): Cortison schädigt selektiv die Fibroblasten in Kulturen mit verschiedenen Zellarten, Maus. Dougerthy (1951): Cortison verhindert im lockeren Bindegewebe der adrenalektomierten Maus die inflammatorische Reaktion auf Histamin. Meier und Gross (1951): Cortison führt zur Änderung der Zellfunktionen vorwiegend im Mesenchym und verhindert die typische entzündliche Gewebereaktion gegenüber bestimmten Reizen. Shapiro *et al.* (1951): Cortison verringert Granulationsgewebe um Terpentinabszesse, Ratte. Ragan (1952): Cortison verhindert Fibroplasie. Germuth (1956): Cortison unterdrückt die zelluläre Antwort nach Traumen der verschiedensten Art. Meier und Desaulles (1957): Cortison hat in Versuchen an der Ratte vorwiegend eine Wirkung auf die mesenchymale Zellreaktion. Diraimondo und Forsham (1958): Elektronenmikroskopische Studien zeigen akute Veränderungen der Fibroblastenstruktur nach Gaben von Hydrocortison in Tierversuchen. Pintar (1960): Glukokortikoide müssen in mehr als therapeutischer Dosis gegeben werden, um die Fibroplasie zu verzögern. Sandberg (1963): Cortison vermindert den Kollagengehalt im Granulationsgewebe, Ratte, Kaninchen.

Die Adhäsionseinschränkung durch Glukokortikoide

Die Bildung von Verwachsungen ist eine natürliche Reaktion des Körpers und speziell der Bauchhöhle auf *Traumen* verschiedener Art und dient der Natur als eine Art von Verteidigungswaffe.

sere Wirkung erzielt als die parenterale (Meier *et al.* 1950 Shapiro *et al.* 1951 Ekeström, 1952 Luttwak *et al.* 1957 Zicha *et al.* 1961 Fruhman, 1962)

Die Glukokortikoidwirkung auf das Mesenchym ist abhängig von der Dosis (Howes *et al.* 1950 Meier *et al.* 1950 Dougerthy 1951 Meier *et al.* 1956) Nach Diraimondo und Forsham (1958) fordert eine Therapie, die das Bindegewebe hemmen soll, die höchstmögliche Dosierung. Pintar (1960) meint, die therapeutische Dosis der Glukokortikoide reiche nicht aus, um die Fibroplasie zu hemmen.

Eine wichtige Tatsache ist, dass Glukokortikoide nicht dieselbe Wirkung auf Epithel und Mesenchym haben. Nach v Brunn (1901) und Efskind (1940) ist die Serosadeckzelle oder das Mesothel genetisch, morphologisch und potentiell als typisches Epithel zu betrachten. Die Beobachtungen verschiedener Autoren sprechen dafür dass die Epithelialisierung durch Kortikosteroide nicht oder weniger als das Bindegewebe gehemmt wird (Howes *et al.* 1950 Plotz, 1950 Spain *et al.* 1950 Alrich, 1951 Billingham *et al.* 1951 Pintar 1960)

Bereits nach 14 Stunden (Roloff 1900) oder 2 Tagen (Hinsberg, 1900) zeigen kleine Fremdkörper in der Bauchhöhle einen Epithelüberzug Graser (1888) beschreibt, dass ein geschädigtes Oberflächenepithel sich nach etwa 4 Tagen regeneriert hat. Williams (954 1955) sieht bei seinen Versuchen dass Wundflächen in der Bauchhöhle bereits nach 3, 5 oder 6 Tagen einen glatten und spiegelnden Überzug haben. Ellis (1962) teilt mit, dass nach 7 Tagen grosse Serosadefekte mit Epithel bedeckt waren. Die Epithelialisierung in der Peritonealhöhle geschieht nicht von den Wundkanten aus, sondern gleichmässig über die ganze Wundfläche sowohl im Tierversuch wie auch beim Menschen (Brunschwig und Robbins, 1953 Howes, 1963 Williams 1954 1955)

Nach drei Stunden findet man bereits feste Verklebungen (Trompke und Siegner 1956) Zu ähnlichen Befunden kommt Eskeland (1963 b) Zaporozhets (1964) berichtet über Adhäsionen nach 8–12 Stunden. Wywodzoff (1867) beschreibt feste Verklebungen nach 12 Stunden. Wereschinski (1925) beobachtet schon nach 24 Kawasoye (1913) nach

schreiben nach parenteraler Cortisonzufuhr beim Hund eine bedeutende Herabsetzung der Verwachsungen in sowohl qualitativer wie quantitativer Hinsicht Eine völlige Adhäsionsfreiheit durch parenterale Cortisongaben erreicht Lyall (1953) bei seinen Versuchen an Meerschweinchen. Zachariae (1954 1955) arbeitet mit intraperitonealen Gaben von Hydrocortisonacetat und hat gute Erfolge bei ihren Versuchen an Kaninchen. Ähnliche Resultate erhalten de Sanctis und Mitarbeiter (1955) bei ihren Versuchen an Hunden. Haße und Speth (1956) finden, dass bei lokaler Applikation das Hydrocortisonacetat ebenfalls bei Meerschweinchen einen sehr guten Effekt in der Adhäsionsprophylaxe hat 1959 kommt Thomaschek mit einer grossen Ver suchsserie an Ratten in der er zeigt dass in 56 % der Fälle postoperative Verwachsungen durch intraabdominale Hydrocortisongaben verhindert werden können. Ehlers und Grimsehl (1960) bestätigen die guten Resultate von Thomaschek. Auch Eskelands (1963 a) Versuche weisen in die gleiche Richtung

Eine Adhäsionsprophylaxe mittels Glukokortikoiden beim Menschen wird von Odell und Mitarbeitern (1951) Haße (1958) Zachariae und Zachariae (1956) Virgili Marques (1960) Käser und Iklé (1961) Rust (1962) und Levy und Ducasse (1965) mitgeteilt. Wie man aus der Literatur und persönlichen Gesprächen mit Gynäkologen in Europa entnehmen kann werden in zunehmendem Masse Kortikosteroide gerne von Operateuren bei Fertilitätsoperationen und Tu barplastiken zur Adhasionsprophylaxe herangezogen sowohl parenteral wie auch lokal (Green Armytage 1960 Palmer 1960 Shirodkar 1960 Japhet 1963) Die Dosierungen hal ten sich jedoch alle in sehr niedrigen – man ist versucht zu sagen, theoretisch eigentlich unwirksamen – Grenzen

Betrachtungen zur Prophylaxe der postoperativen Verwachsungen mit Hilfe von Glukokortikoiden

Aus der zur Verfugung stehenden Literatur geht eindeutig hervor dass in Hinsicht auf die Bindegewebs und Inflamma tionshemmung die lokale Applikation von Kortikoiden eine bes

Anwendung von sehr grossen Dosen Glukokortikoid für die intraabdominale Adhäsionsprophylaxe dienen.

Eigene Versuche

Versuchstiere und allgemeine Versuchsbedingungen

Die Versuche wurden an 8 Primaten der Unterfamilie Cercopithecinae (Asdell, 1964) in Gruppen zu je 2 Tieren in der Zeit vom 25.2.1962 bis zum 27.9.1965 durchgeführt. Die erste Gruppe waren 2 Tiere der Art Macaca cynomolgus (Süd-Ostasien) mit 2,5 bzw. 3,5 kg Gewicht, alle übrigen Tiere waren von der Gattung Cercocebus Mangabe (Westafrika) mit einem Gewicht von ungefähr 6 kg. Die Tiere waren zu zweit in grossen Spezialkäfigen auf der Tierabteilung des virologischen Institutes untergebracht. Die durchschnittliche Quarantänezeit war etwa 2-3 Wochen, während der ersten 10 Tage nach der Ankunft bekamen die Tiere täglich 12 mg Aureomycin® aufgelöst in Milch. Das Futter bestand morgens aus Weissbrot und Milch mit Zugabe eines Polyvitaminpräparates, mittags aus gekochten Eiern, gekochten Kartoffeln und Knäckebrot und abends aus Bananen, Apfelsinen, Äpfeln und Mohrrüben, dazu Trinkwasser in beliebiger Menge.

Narkose und Operationsmethodik

Die Narkose bot gewisse Schwierigkeiten. Die Prämedikation geschah immer mit Lergigan® und Phenemal, wovon die Tiere erstaunlich grosse Dosen vertrugen. – Zum Beispiel bekamen die Tiere der letzten Gruppe mit relativ kleinen Mangaben 70 mg Lergigan® und 140 mg Phenemal per injectionem intramuscularem 1 ½ Stunde vor der Einleitungsnarkose, ohne dass die Tiere hierdurch besonders schläfrig wurden. – Nach einer intramuskulären Einleitungsnarkose mit 0,25 mg Atropin und 200 mg Evipan® wurde bei den Mangaben eine Intubationsnarkose mit Spontanatmung und Stephen-Slaterventil (non-rebreathingsystem) durchgeführt, die Cynomolgusgruppe erhielt eine parenterale Barbituratnarkose.

Nach Rasieren, Waschen und Desinfektion der gesamten

48 Stunden und Graser (1888) nach 3 Tagen eine Vaskularisation von Verwachsungen. Sowohl Thomaschek (1959) wie auch Ehlers und Grimsehl (1960) teilen mit, dass sich das Adhäsionsbild nach drei Tagen nicht mehr ändert. Nach Thomas und Rhoads (1950) ist die Adhäsionsbildung nach 3–6 Tagen gewöhnlich abgeschlossen. Aus den Versuchen von Eskeland (1963 b) geht hervor dass die exsudative Phase nach einem Peritonealtrauma nicht länger als drei Tage währt.

Die Glukokortikoidwirkung ist im Hinblick auf die Adhäsionsprophylaxe am besten – oder vielleicht nur so effektiv – wenn eine Applikation vor der oder im direkten Anschluss an die Operation geschieht (Kosdoba 1934 Spain *et al.* 1950 Odell *et al* 1951 Carstam 1953 Eskeland 1963 a, Sandberg 1963 Boggs *et al.* 1964)

Aufgrund der oben erwähnten Daten und Befunde können folgende Schlussfolgerungen für die Anwendung von Glukokortikoiden zur Adhäsionsprophylaxe gezogen werden

a) Die Anwendung muss vor allem lokal das heisst in unserem Fall intraperitoneal erfolgen
b) Die Dosierung muss sehr hoch sein.
c) Die Applikation muss kurz vor oder während der Operation erfolgen.
d) Die Medikation kann kurzzeitig sein.

Das Risiko von unerwünschten Nebenwirkungen wird durch die unter a) und d) genannten Forderungen sehr verringert. Der Sinn einer Adhäsionsprophylaxe mit Hilfe von Kortikoiden ist, kurz gesagt eine Epithelialisierung zu erreichen bevor die Bindegewebsreaktion mit der nachfolgenden Adhäsionsbildung ein setzt.

Will man die zitierten Tierversuche auf den Menschen übertragen so bedeutet das wenn man beispielsweise die nach Thomaschek (1959) für eine Ratte optimale Dosis von 10 mg Hydrocortison als Richtschnur nimmt eine Menge von rund 3000 mg Hydrocortison für eine Frau von etwa 60 kg Gewicht. Die an Primaten geplanten Grundversuche mit einer Resektion der Tuba Fallopii sollen gleichzeitig als Test für die klinische

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Nach Rasieren, Waschen und Desinfektion der gesamten

Bauchhaut wurde unter sterilen Kautelen die Operation mit einem infra umbilikalen Mittellinienschnitt begonnen. Die stets sehr grosse und wohlgefüllte Blase wurde durch Punktion mit einer feinen Nadel entleert da es nicht möglich war die Tiere zu katheterisieren. Die Punktionsstelle wurde mit einer zweifachen Tabaksbeutelnaht (000 00-Catgut) geschlossen. Operationsmikroskop oder Lupenbrille erleichterte das Arbeiten mit den feinen Strukturen. Jedes Tier bekam im Anschluss an die Operation eine intramuskulare Injektion von 25 mg Achromycin® und danach fur einige Tage 12 mg Aureomycin® mit der Morgenmilch

Die Operationsmethodik war im Prinzip folgende Bei allen Tieren wurde bilateral ein ½–1 cm langes Stück aus dem mittleren Drittel der Tube reseziert, wobei sorgfaltig darauf geachtet wurde dass die Resektionen bei ein und demselben Tier gleich lang waren Bilateral wurde der Mesosalpinxschlitz mit einer atraumatischen Knopfnaht (000 00) geschlossen. Die Resektionsstelle der einen Tube wurde mit Hilfe einer einzigen atraumatischen Knopfnaht (000 00 oder 000 000-Catgut) auf der antimesenterischen Kante vereinigt Es wurde immer versucht eine sero-muskuläre Naht zu legen. Die Tube der anderen Seite wurde ohne Naht gelassen. Meistens wurde eine Markierung der Resektionsstellen auf beiden Seiten mit einer atraumatischen Knopfnaht aus synthetischem nicht resorbierbarem Material (000 00-Dermalene® Davis & Geck) durchgeführt. Vor Schluss der Bauchwunde erhielt jedes Tier eine Nahtreihe mit je einer Seiden Catgut und Dermalenenaht (Starke 000 00 mit atraumatischer Nadel) mit ½ cm Abstand, um spater die Reaktion des Gewebes auf das verschiedene Nahtmaterial histologisch untersuchen zu konnen (Tier I und II im Blasenfundus Tier III–VIII seitwärts der Inzision unter die Bauchdecke) Der Verschluss der Bauchdecken wurde immer wie folgt ausgefuhrt Fortlaufende Catgutnaht (000) in Peritoneum und Muskulatur Naht der Fascia mit Knopfnähten (Seide 000) und Hautnaht mit einzelnen Knopfnähten (abwechselnd Seide 000 und rostfreier Stahl 000) Wundverband mit Hilfe von Nobecutan Spray® (flussiger gewebefreundlicher Plastverband der Firma Bofors) und Mullbinden.

Das erste Tier in jeder Gruppe erhielt vor Schluss der Laparo-

tomiewunde eine Instillation von Glukokortikoid intraperitoneal (fossa Douglasii) wobei darauf geachtet wurde dass das Steroid sorgfältigst über das gesamte Operationsfeld verteilt wurde. Abgesehen von Affe I der 2,5 kg wog und 1 ml = 25 mg Ledercort® (Triamcinolon, Lederle) bekam, erhielten die Tiere III, V und VII 12 ml = 300 mg Hydrocortodrin® (Hydrocortisonacetat, Astra)

Reoperation und Nachkontrolle

Die Reoperation geschah nach 10 (Gruppe 1) 8, 6 oder 4 (Gruppe 4) Wochen. Nach einer letalen Dosis Barbiturat wurde der gesamte Bauch durch einen paramedianen Längsschnitt geöffnet und der Situs aufgezeichnet und fotografiert. Darauf wurde der Uterus mit Adnexen herauspräpariert um die Durchgängigkeit der Tuben mit Hilfe von mit Methylenblau gefärbter Kochsalzlösung und der Hysterosalpingographie zu prüfen. Zur Injektion wurde eine in die Cervix oder Vagina eingebundene Knopfkanüle benutzt

Die technischen Daten für den grössten Teil der Röntgenbilder sind folgende Film Kodak Crystallex Kontrastmittel Biligrafin B forte® 50 % Belichtungswerte 300 mA, 35 kV 2 5 sec, Fokus-Filmabstand 110 cm, Fokusgrösse der Röntgenröhre 0 6×0 6 mm. Nach der Röntgenuntersuchung wurden die Uteri mit Adnexen sofort für die spätere histologische Untersuchung in Formalin gelegt.

Ergebnisse

Alle Operationswunden heilten per primam Die postoperativen Verläufe waren komplikationsfrei – soweit man aus dem normalen Verhalten der Tiere darauf schliessen darf – die Tiere frassen und tranken am ersten postoperativen Tage mit Ausnahme von Tier I das noch fast den ganzen Tag schlief Abgesehen von den später näher zu beschreibenden Verwachsungen boten alle Eileiter einen normalen Aspekt, es fanden sich weder Dehiszenzen noch überschiessende Wucherungen von Narbengewebe oder Mukosa. In allen Gruppen, das heisst also auch bereits nach 4 Wochen (Gruppe 4) bot sich das Bild einer abgeschlossenen Heilung, sowohl bei den Tieren mit intraperito-

nealer Steroidapplikation (I, III V VII) als auch bei den Tieren ohne Glukokortikoide (II IV VI VIII)

Bei der Kontrolle der Durchgängigkeit fand sich in dem genahten rechten Eileiter des Tieres II keine Passage, weder bei Spulung mit Methylenblau noch bei der Hysterosalpingographie. Man konnte deutlich beobachten, wie die blaugefärbte Kochsalz lösung bis zur Stelle der Anastomose gelangte das Röntgenbild verifizierte darauf diesen Befund. Das Tier VI hatte Verwachsungen zwischen der Blase und den rechten Adnexen die die Markierungsnaht verdeckten und es ausserdem schwer machten, den Verlauf des lateralen Teils der Tube zu verfolgen. Erst beim vierten Versuch zeigte sich sowohl bei der Spulung wie auch bei der Röntgenkontrolle eine Passage über die Anastomose hinaus. Zu bemerken ist hier dass wahrend der Operation von Tier II (auf der zu nähenden, rechten Seite) und von Tier V (auf beiden Seiten) recht beschwerliche Blutungen von den Resektionsstellen die Operation komplizierten. Bei Tier II musste ausser der geplanten Naht der Tube an der antimesenterialen Seite noch eine Umstechung der Tubarkante nahe der Mesosalpinx gemacht werden. Im Falle des Tieres V standen die Blutungen auf beiden Seiten nach der Naht der Mesosalpinxschlitze und einer mehrere Minuten dauernden, manuellen Kompression. Eine Zusammenstellung des Verhaltens der Eileiter in bezug auf die Durch gängigkeit gibt die Tabelle Nr I Die Abbildungen 1 a und 1 b zeigen fotografische Wiedergaben zweier Hysterogramme in natürlicher Grosse.

Die Tiere die eine intraabdominale Kortikoidapplikation erhalten hatten (I III V VII) zeigten deutlich eine Verringerung der Zahl und der Ausbreitung der Verwachsungen. Affe III und VII hatten überhaupt keine Adhasionen. Ein zarter segelförmiger etwa ½ cm breiter Verwachsungsstrang fand sich bei Tier I zwischen der kaudalen Bauchnarbe und dem Omentum. Affe V hatte eine fast haarfeine dünne Verwachsung an der gleichen Stelle die sich bereits spontan bei Anheben der Bauchdecken zwecks Inspektion der Bauchhohle löste. Dieses Tier hatte wahrend der Primäroperation reichlich geblutet hier hatten sich während der Operation bereits nach kurzer Zeit zahlreiche und recht zähe Fibrinfäden gebildet die dann mit Hilfe von feuchten



Abb. 1



Abb. 1b

Abbildung und b Postmortale Hysterosalpingogramme von Cercocebus Mangabe 6 Wochen (Aff V Abb) und 8 Wochen (Aff IV Abb. b) nach bilateraler Resektion aus dem mittleren Drittel der Eileiter Natürliche Grösse.

Tabelle I. *Durchgängigkeitsprüfung der Eileiter mit Hilfe von gefärbter Kochsalzlösung (Spülung) und Hysterosalpingographie (HSG)*

Tier N	Intervall für Reoperation	genähte Tube		nicht genähte Tube	
		Spülung	HSG	Spülung	HSG
I (Kort.)	10 Wochen	+	+	+	+
II		—	—	+	+
III (Kort.)	8 Wochen	+	+	+	+
IV		+	+	+	+
V (Kort.)	6 Wochen	+	+	+	+
VI		(+)	(+)	+	+
VII (Kort.)	4 Wochen	+	+	+	+
VIII		+	+	+	+

Eine Kortikoidapplikation während der Operation wird in der Klammer hinter der Nummer des Tieres angegeben

Kompressen gelöst wurden. Eine etwa doppeltlinsen grosse, weisse Ablagerung (siehe Abbildung 2 a) die mit aller Wahrscheinlichkeit ein Steroidrest ist fand sich bei Tier VII sowohl in der excavatio vesico-uterina als auch in der excavatio recto-uterina

Bei den nicht mit Glukokortikoid behandelten Tieren (II IV VI VIII) die die Kontrolltiere für die Versuche betreffend die intraperitoneale Steroidapplikation darstellten fanden sich in jedem Falle Verwachsungen, was mit den Befunden Gepferts (1939) übereinstimmt der in seinen Versuchen an Macaca Mulatta in allen Fällen extensive peritubare Verwachsungen beschreibt. Die Verwachsungen hatten folgende Ausmasse Tier II Omentum fixiert an der gesamten Bauchnarbe und dem oberen Blasenzipfel mittelschwere knapp 1 cm breite Verwachsungsstränge zu den rechten Adnexen, dem Uterusfundus und dem oberen Rectum, ähnlicher Strang zwischen Blase und linker Tube Tier IV Bauchnarbe und Blasenzipfel wie II feiner Strang zur rechten Tube gröberer Strang zwischen linker Tube und dem Colon.

Tabelle II. *Umfang und Ausmass der intraperitonealen Verwachsungen aufgeteilt in Tiere mit und ohne intraperitoneale Glukokortikoidapplikation.*

Tier Nr. und Op.-Datum	Intervall der Kompression	mit Kortikoid	ohne Kortikoid	Bemerkungen
I (25.5.62)	Wochen	+		
II (5.6.62)			++	Untersuchung wegen Blutungen auf der re. d.h. genähten Seite
III (3.1.63)	8 Wochen	0		
IV (19. 63)			++	
V (23. 64)	6 Wochen	(+)		Kräftige Blutungen bilateral. Blutstillung spontan nach Kompression
VI (16.1.64)			++	
VII (17.8.65)	4 Wochen	0		
VIII (30.8.65)			+++	

Einteilung der Verwachsungen in: leichte +, mittelschwer ++, schwere +++

Eine minimale Verwachsung ist mit (+) bezeichnet.

Tier VI, segelförmige Adhäsionen am grössten Teil der Längsschnittnarbe an der vorderen Bauchwand, Blase verwachsen mit der Vorderseite der rechten Adnexe, ein feiner Strang von der lateralen linken Tube zur Beckenwand. Tier VIII, vordere Bauchwand wie VI, ausserdem nicht zu dünne Verwachsungen vom Oment zum unteren Sigmoideum und rechter Tube samt Ovarium (siehe Abbildung 2 b, 2 c). Eine Zusammenfassung der Resultate

betreffend die Adhäsionsbildung mit und ohne intraperitoneale Kortikoidapplikation gibt die Tabelle II Die Adhäsion im Fall I *war leicht und wird mit +* bezeichnet, wenn man der Einfachheit halber eine Aufteilung der Adhäsionen in leichte + mittel schwere ++ und schwere +++ vornimmt. Die Adhäsionen *in den Fällen II, IV und VI waren mittelschwer und erscheinen* in der Tabelle als ++ Die Verwachsungen im Fall VIII wurden als schwer bezeichnet und sind mit +++ angegeben. Die Adhäsion im Fall V *wurde als minimal eingestuft und* ist in der Tabelle mit (+) aufgeführt

Diskussion der Ergebnisse

Aus den obengenannten Befunden geht hervor dass eine Querresektion der Eileiter bereits nach 4 Wochen geheilt ist. Die Tuba Fallopii hat eine gute Heilungstendenz und neigt nicht zu Fistelbildung Die Kontinuität der Tube stellt sich auch ohne Naht der Tubenwand wieder her Es scheint so als ob die Tubenwände die Fähigkeit hätten, nach einer glatten Querresektion spontan den entsprechenden Strukturen der gegenüberliegenden Tubenkante entgegenzuwachsen Eine Querresektion der Tube scheint nicht – oder zumindestens selten – im Vergleich mit dem Darm zu einer Stenosierung infolge von Schrumpfung oder Hypertrophie von Muskulatur oder Bindegewebe zu führen. Die Stenosierung im *Fall II auf der Seite der genähten Tube dürfte auf die Blutung* mit nachfolgender Umstechung zurückzuführen sein. Alle diese Eigenschaften der Tuba Fallopii dürften für einen an der Tubarchirurgie interessierten Operateur wissenswert sein

Die Naht der Tube mit einer vielleicht nicht ganz exakten Adaption der verschiedenen Gewebsanteile und der darauf folgenden Reaktion des Gewebes auf den eingeführten Fremdkörper (Gepfert 1939) stellen sicherlich ein gewisses Risiko bei einer Operation der Tuba Fallopii dar In diese Richtung weist sowohl der eben zitierte Fall II wie auch die relative Passagebehinderung im Fall VI – Trotz der geringen Dicke einer sehr feinen atraumatischen Catgutnaht (000 000 bei Tier VI) wirkt diese bei einer Betrachtung im Operationsmikroskop wie ein grobes Seil im Vergleich mit dem *minimalen Tubarlumen eines kleinen Affen.* –

Die intraperitoneale Applikation von Glukokortikoiden direkt im Anschluss an die Operation, das heisst mit anderen Worten vor Schluss der Bauchdecken, verringert eindeutig die Zahl und Ausbreitung der Adhäsionen nach einer Operation an gesunden Eileitern bei Primaten. – Der anfangs beabsichtigte Plan einer Operation an vorher mit einer experimentellen Salpingitis be lasteten Primateneileitern musste aufgrund von zu grossen Schwierigkeiten fallen gelassen werden. – Dies steht in gutem Einklang mit früher publizierten Resultaten bei anderen Tieren (siehe oben Adhäsionseinschränkung durch Glukokortikoide) Weil ein grösseres Volumen des applizierten Glukokortikoids wünschenswert war wurde bei den Tieren III V und VII Hydrocortison anstelle von Triamcinolon verwendet. Die sehr grossen intraperitonealen Steroiddosen haben zu keiner Wundkomplikation geführt, sie haben ausserdem keinen schädigenden Einfluss auf die Heilung der resezierten Tuben bei einer postoperativen Kontrolle innerhalb von 4–10 Wochen. Die von einigen Autoren (Ekeström, 1952 Hubay et al. 1953 Thomaschek, 1959 Eskeland, 1963 a) beschriebene grössere Empfindlichkeit fur Infektionen im Zusammenhang mit Glukokortikoidapplikation bei Operationen ist auch vom Verfasser selbst bei Versuchen mit kleineren Tieren beobachtet worden und dürfte sich vielleicht durch die erheblichen Schwierigkeiten erklären, die die Durchführung von Tieroperationen unter sterilen Bedingungen macht.

Eine bisher unüberwindliche nicht beabsichtigte, doch anscheinend völlig ungefährliche Nebenwirkung der intraperitonealen Kortikoidapplikation besteht in der Tendenz der Steroidkristalle sich zu postoperati en Ablagerungen (Thomaschek, 1959) zusammenzuballen (siehe Abbildung 2 a)

Wohl bewusst der Tatsache dass die oben beschriebenen Versuche an gesunden Tuben von Primaten durchgeführt wurden, kann man daraus etwa folgende Schlüsse ziehen, die auch für die Chirurgie der menschlichen Eileiter nicht ohne eine gewisse Bedeutung sein dürften Eine feste Vereinigung der Resektionskanten der Tube die eine Belastung wie z.B Spulung und Hysterosalpingographie erträgt, ist bereits nach 4 Wochen eingetreten, hieran ändert auch eine sehr hoch dosierte intraperitoneale Glukokortikoidapplikation zur Zeit der Operation nichts – Das

Epithel der Tube hat eine gute Heilungstendenz und neigt nicht zu Fistelbildung (nähere histologische Einzelheiten werden in einer folgenden Arbeit besprochen) Eine sehr sparsame Anwendung von Nahtmaterial ist bei der Operation von Eileitern zu empfehlen da anscheinend eine Art von Taxis der einzelnen Schichten der Tube – oder vielleicht nur des Epithels? – besteht die dazu fuhrt, dass die Resektionskanten der Tuba Fallopii nach einer Querresektion auch ohne Naht wieder anstandslos miteinander verwachsen können. Eine Naht mit nicht exakter Adaption ist vielleicht schädlicher für eine gute Rekanalisierung als eine fehlende Naht.

Aufgrund der beschriebenen Versuche an Affen erscheint es sinnvoll und ungefahrlich, eine intraperitoneale Applikation von sehr grossen Dosen Glukokortikoid fur die klinische Adhäsionsprophylaxe bei plastischen Operationen an den Eileitern zu versuchen.

ZUSAMMENFASSUNG

Es werden Querresektionen der Tuba Fallopii an Primaten der Unterfamilie der Cercopithecinae beschrieben, bei denen nach einer bilateralen Resektion nur die Tubenwande der einen Seite durch eine Naht vereinigt wurden, während die Tubenwände der anderen Seite einer Spontanheilung ohne Naht uberlassen wurden. Die Halfte der Tiere erhielt eine sehr grosse intraabdominale Glukokortikoidapplikation am Schluss der Operation. Die Reoperation geschah mit einem Intervall von 4–10 Wochen. Die Kontrolle der Durchgängigkeit mit Hilfe von Farblösung und Hysterosalpingographie bewies dass eine gute Spontanheilung auch ohne Naht der Tube eintritt sogar in den mit Kortikosteroiden behandelten Fällen selbst nach einem Intervall von nur 4 Wochen. *Die intraabdominale Kortikoidapplikation* verminderte die Anzahl und das Ausmass der postoperativen Adhasionen und führte zu keinen Komplikationen.

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SUMMARY

A bilateral resection of the middle portion of the Fallopian tubes was carried out on 8 monkeys. The purpose of the study was to find out if suturing of the tube is necessary for recanalization, and if large intraperitoneal doses of glucocorticoids have any effect on the healing process and on the development of postoperative adhesions. In each animal the tubal wall on one side was left unsutured while on the other side the resection edges of the tube were connected by means of one single exact stitch. Every second animal got an intraabdominal instillation of very high doses of glucocorticoids at the end of the operation. Reoperation was per formed after an interval of 4 6 8 or 10 weeks. The recanaliza tion was controlled by means of hydropertubation and hystero-salpingography

Spontaneous recanalization was found in all cases where the resected tube was left without suturing The intraperitoneal application of glucocorticoids at the end of the operation clearly reduced the extent of postoperative adhesions and did not in volve any complications.

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EXPERIMENTELLE STUDIEN ZUR PROPHYLAXE VON INTRAABDOMINALEN VERWACHSUNGEN

Versuche an der Ratte mit einer Emulsion aus Lipid d Prednisolon

VON

KURT SWOLIN

Verwachsungen und Verklebungen sind ein grosses – wenn nicht sogar das grösste – Problem bei Fertilitätsoperationen und Tubarplastiken (Holden und Sovak 1932 Gepfert 1939 ten Berge und Tik Lok, 1954 Rock *et al.* 1954 Green Armytage 1960 Vara, 1959 Caplier 1964 Swolinzky 1965 Palmer 1966) Nicht nur für den Gynäkologen, sondern auch für den Chirurgen sind Adhäsionen ein sorgenvolles Kapitel (Payr 1924 Lyall 1953 Wangensteen 1955 Connolly und Smith, 1960 Matsumoto 1964 Powley 1965) Riedel (1941) hat das einmal kurz und treffend ausgedrückt Wo Verwachsungen sind kommen auch wieder welche hin. Dieser Ausspruch hat im grossen und ganzen leider auch heute noch immer Gültigkeit. Die Tabelle I gibt eine Übersicht über die Häufigkeit von postoperativen Adhäsionen.

Payr (1914) der sich ein ganzes Leben lang mit Adhäsionsproblemen beschäftigt hat, sagt an Hand eines eigenen grossen Materials dass 12–15 % der laparotomierten Patienten typische Adhäsionsbeschwerden haben und dass 3,2 % aufgrund von starken Beschwerden einmal oder mehrmals reoperiert werden. Ehrler (1961) schätzt, dass es auf 100.000 Einwohner etwa 15.000 potentiell Adhäsionsträger gibt, wovon 10–20 % im Laufe ihres Lebens behandlungsbedürftig werden. Gynäkologische Operationen prädisponieren anscheinend mehr für Verwachsungen und

[illegible]

Tabelle I *Die Häufigkeit von postoperativen Adhäsionen*

Verfasser	Adh. in %	Material	Fremdkörp.
Martin 1888	91	22 Relaparotomien	gyn.
Naegeli 1919	79	Pneumoperiton (42)	–
Dorsett 1920 1921	80	100 Relaparotomien	
Mayer 1922	87	60 Relaparotomien	gyn.
Haug und Heudorfer 1923	89,3	140 Pat.op in fremder Klinik, Relap	gyn
Haug und Heudorfer 1923	83,4	96 Pat.op in eigener Klinik, Relap.	gyn.
Turunen 1933	86,9	1910–1931 Univ Frauenklin. Relap	gyn.
Kaufman 1935	88	509 Relaparotomien	–
Duff 1939	80–90	Pneumoperiton. (148)	–
Burger 1941	73	10-Jahresmaterial Univ -Frauenklin Relaparotomien	gyn
Green 1946	96	50 Relaparotomien	–
Benzer et alii 1964	71,8	303 Obduktionen	–

Ileus oder haben anders ausgedruckt eine grössere Tendenz, von postoperativen Adhäsionen gefolgt zu werden (Dorsett, 1920 1921 Haug und Heudorfer 1923 Clairmont und Meyer 1929 Turunen 1933 Kaufman, 1935 Burger 1941 Becker 1952 Miller und Winfield, 1959 Wist 1962 Backlund und Fries 1964 Weiss *et al* 1964) Es ist nicht die Aufgabe dieser Arbeit die Ursachen hierfur zu diskutieren es ist dagegen wunschenswert und an der Zeit, diese vor allem im Hinblick auf die Fertilitätsoperationen bedauernswerte Tatsache einmal deutlich zu pointieren.

Nach Christopher (1941) sind 30–40 % aller Fälle von intestinaler Obstruktion durch Verwachsungen verursacht, ähnliche Zahlen findet man bei anderen Autoren Flesch Thebesius (1920) 44 % McIver (1932) 30 % Perry *et al* (1955) 31 % Powley (1965) 40 %

Die Mortalitätsziffern fur Adhasionsileus waren und sind noch immer erschreckend hoch. Sie sind nach neuesten Mitteilungen (Powley 1965) durchaus nicht im Abnehmen im Gegenteil nach einer deutlichen Abnahme in den 40 iger Jahren zeigt die

Kurve der Mortalitätsziffern aufgrund von Adhäsionsileus wieder eine steigende Tendenz, im Gegensatz zur Mortalität nach Appendicitis oder Hernie. Nach Dunkel (1935) hat 1 % aller gynäkologischen Operationen einen Ileus zur Folge. Häggström (1926) nennt sogar eine Zahl von 1 % Ileus nach Kaiserschnitten, wobei anzumerken ist, dass sowohl im Tierexperiment wie in der Klinik eine Gravidität die Adhäsionsbildung deutlich vermindert (Burger 1941 Seeley 1942 Levy und Ducasse 1965) Die Tabelle II gibt einige Literaturhinweise auf die Mortalitätsfrequenz infolge von Adhäsionsileus.

Tabelle II *Mortalität im Zusammenhang mit Adhäsionsileus*

Verfasser	Mortalität in	Anmerkung
Pay [illegible]	7,8	postop. und spontane Adhäsionen
Burgess [illegible]	37,0	
Souttar [illegible]	33	
McIver [illegible]	44	
S-Krook 947	[illegible]	Spätileus Dünndarm
Becker 952	[illegible]	
Perry et al. 955	9,5	
Müller und Winfield 959	7,0	postop. Adhäsionen
Backlund und Fries 964	[illegible]	
Weiss et al. 964	[illegible]	postop. Adhäsionen
Powley 965	6,7	Dünndarm, Erwachsene

Die obengenannten Zahlen lassen deutlich verstehen, warum Chirurgen und Gynäkologen sich seit vor der Jahrhundertwende intensiv mit den Problemen der postoperativen Verwachsungen befasst haben. Die Literatur ist voll von Mitteln und Methoden die zur Adhäsionsprophylaxe vorgeschlagen worden sind. Die folgende Aufzählung mag einen ungefähren Einblick in die Vielzahl der vorgeschlagenen Mittel vermitteln.

Allantoismembrane (G p f t, [illegible] Aff.) Aluminiumhydroxyd (T[illegible] [illegible] 963, Kaninchen) Ammoniakonzentrat (J b [illegible], 925, homo) Chondroitin und Polyvinylpyrrolidon (M t [illegible] t 964 homo) Cortison (Z h [illegible] und Z h [illegible] 956 homo intraperitoneal) Dextran (C h [illegible] et al. 964 Ratte) Dicumarol (W h t 949, Hund) Elektromagnet Fremdkörper im Magen-Darmkanal (F y [illegible] 3 homo) Blockierung des elektrischen Potential (C [illegible] und S [illegible] 93 Kanin-

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Fey [illegible]4	7,8	postop. und spontane Adhäsionen
Burgess 1929	37,0	
Souttar 1929	33	
Mc Iver 1932	44	
S-Krook 1947	20	Spätileus Dünndarm
Becker 1952	,8	
Perry et alii 1955	9 5	–
Miller und Winfield 1959	7,9	postop. Adhäsionen
Backlund und Fries 1964	8	
Weiss et alii 1964	,2	postop. Adhäsionen
Fowley 1965	16 7	Dünndarm, Erwachsene

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Allantoismembrane (G[illegible]pf[illegible]rt 1939 Affe) Aluminiumhydroxyd (T[illegible]a, 1965, Kaninchen) Ammoniumkonzentrat (J[illegible]h[illegible]n, 1928, homo) Chondroitin und Polyvinylpyrrolidon (M[illegible]um[illegible]o, 1964 homo) Cortison (Z[illegible]h[illegible] und Za[illegible]h[illegible] 1956 homo intraperitoneal) Dextran (Ch[illegible]t et al. 1964, Ratte) Dicumarol (Wh[illegible]t 1949 Hund) Elektromagnet Eisenpulver im Magen-Darmkanal (Pay[illegible] 19[illegible]7 homo) Blockierung des [illegible]elektrischen Potential (C[illegible]n und S[illegible]u, 193[illegible] Kanin-

chen) Fett humanes [Humanol] (Eden und Lindig, 1920 Ratte, Katze, homo) *feuchte Tücher* (Walthard 1893 Kaninchen Katze] Fibrinolysin, humanes [Actase der Firma Ortho] (Villavicencio und Gross, 1962 Hund, I v guter Effekt intraperitoneal kein Effekt) Glaskörpermasse vom Kalbsauge (Pribram 1914 Kaninchen Hund) *Gummi arabicum* (Vogel, 1917 Kaninchen, homo) Heparin (Lehman und Boys, 1940, Kaninchen, *Hund) Hyaluronidase* (Myburgh 1953, Kaninchen homo) intraluminale Intubation (Vadheim und Wickström 1963, homo) Kabikinase (James und Ellis 1964 Ratte kein Kaninchen guter Effekt) Koktol-Kochsalz lösung (Papayotinextrakt) (Kubota 1924, Kaninchen Katze Hund, homo) Kollodium (Stern 1889) Massage externe (Uyeno 1909 Kaninchen) homogenisierte Milch (Kocher 1914 *homo) kampferöl* (Kawasoye 1913 weisse Mäuse Meerschweinchen, Kaninchen) Novocainlösung, intraperitoneal und Hypothermie (Laktionov 1962 Kaninchen) Olivenöl (Martin 1895 homo] Omentektomie (Ehrler 1961 homo) Oment transplantation (McGehee 1942 Hund) Plasminogen bovines und humanes mit Aktivator [Urokinase] (Jewett *et al* 1965, Ratte Kaninchen Hund) Polysiloxanefilm (Cook, 1964 Hund homo) Polyvinylpyrrolidon (Mussgnug, 1956 homo) Polyphloretinphosphat (Backlund und Fries, *1964 homo) Prostigmin und frühzeitiges Essen* (Schiff *et al.* *1950 Hund)* Rheomacrodex (Kern und Kuhbier 1964 Mäuse) Natrium-Rhicinoleat (Seeley 1942 Kaninchen, Affe) Silikonaerosol Silikonröhren (Galen und Cook 1964, Hund) Streptokinase und Streptodornase (Wright *et al.*, 1950, Kaninchen) Trasylol (Kern und Kuhbier 1964, Mäuse) Tripsepar [Trypsin-Heparinkombination] (Laurentaci und Oliva, 1961 Ratte) Vakzinschock (Battezzati *et al* 1964 Kaninchen) Vitamin E (Ruggiero und Sabbatini 1952 Kaninchen) oxydierte Zellulose (Dmytryck, 1948 *Hund)*

Die Vorschlage zur Adhasionsprophylaxe lassen sich in etwa, gemäss ihres *Wirkungsprinzips in einigen wenigen Gruppen zu* sammenfassen (siehe Tabelle III)

Tabelle III *Massnahmen zur Beeinflussung der Adhäsionsbildung*

1 Begrenzung des Peritonealtraumas (z B. Mikrochirurgie)
2 Peritonealisierung (Effekt umstritten)
3 Kontrollierte Adhäsionsbildung (z B Plikationsmethode nach Nobel)
4 Förderung der Darmperistaltik (z B Synstigmininjektion)
5 Omentresektion
6 Blockierung *des elektrischen Potentials*
7 Verhinderung der Fibrinbildung und der Koagulation von Exsudat (z B Heparinapplikation)
8 Beeinflussung der Fibroplastproliferation (z B Cortisonapplikation)
9 Einführen einer Zwischenschicht

Das Einführen einer Zwischenschicht, die die geschädigten Organe oder Gewebe der Bauchhöhle voneinander fernhält, bis die Reperitonealisierung eingetreten ist oder begonnen hat, scheint eine sinnvolle, ja, man möchte sagen, die natürlichste Massnahme zu sein, um Verwachsungen zu vermeiden, da sie sozusagen nicht in den physiologischen Ablauf der Heilungsprozesse einzugreifen braucht. Dies ist deshalb seit Beginn der Adhäsionsforschung in vielen Varianten immer wieder versucht worden. Alle Versuche sind bis jetzt daran gescheitert, dass man einerseits nicht eine Substanz gefunden hat, die einfache Grundforderungen erfüllte die man rein theoretisch an eine solche, sozusagen ideale Trennschicht stellen kann. Andererseits hat es bisher kaum eine Möglichkeit gegeben den Erfolg oder Nichterfolg eines Mittels mit objektiven Methoden an klinischem Material zu beweisen. Dieser Nachteil kann jetzt mit Hilfe der Laparoskopie beseitigt werden (Swolin, 1967) Rein theoretisch lassen sich folgende Forderungen für eine ideale Trennschicht aufstellen

A) Die Substanz darf nicht das Peritoneum reizen und den Körper zu einer örtlichen Verteidigungsreaktion, z.B in Form von Exsudat mit nachfolgender Fibrinbildung und Fibroplasie veranlassen.

B) Die Resorption muss langsam vor sich gehen, damit der Körper in der Zwischenzeit die Möglichkeit hat, eine Reperitonealisierung durchzuführen oder – was vielleicht genügt – zu beginnen.

C) Die Resorption sollte wenn möglich, vollständig sein.

D) Das gewünschte Medium muss effektiv den Kontakt der geschädigten Organe oder Strukturen vermeiden, d.h es muss vor allem an den lädierten Stellen haften oder liegen bleiben, ohne durch eine eventuelle Exsudation oder Lageveränderung entfernt zu werden.

E) Die Trennschicht sollte möglichst dem elektrischen Potential der verwundeten Oberflächen Rechnung tragen (Cantacuzène und Soru, 1931)

Die Forderung unter A) wird leicht von mehreren Substanzen erfüllt. Schwieriger stellt es sich dagegen, eine dem unter B) gestellten Anspruch gerecht werdende Substanz zu finden. Ohne sich in den Streit der Meinungen mischen zu wollen, wo und

wie die in die Bauchhohle eingebrachten Substanzen verschwinden genugt es für die Zwecke dieser Arbeit zu wissen dass im allgemeinen Abfluss und Resorption von der Bauchhohle erstaunlich schnell geschehen (v Recklinghausen 1862 v Dembowski 1888 Muscatello 1895 Steinberg 1944 Courtice und Simmonds 1954 Fries 1956 Pritchard und Weisman 1957) Die Forderung, dass das gewünschte *Medium so lange liegen bleibt bis eine Reperitonealisierung abgeschlossen ist – oder eingesetzt hat – lässt die meisten der vorgeschlagenen Mittel ausscheiden.*

Das körpereigene Fett erbietet sich logischerweise als ein recht ideales die traumatisierten Gewebe trennendes Gleitmittel. An Versuchen hiermit fehlt es natürlich nicht (Eden und Lindig, 1920 Löhnberg, 1922 Inthorn 1938 Biermer 1950) Die Sache hat nur einen Haken die unter B) genannte Forderung wird zu gründlich erfullt. Das Fett sammelt sich oder fliesst in Ölzysten zusammen und wird eventuell spater vom Körper organisiert, so dass die Ablagerungen dann das Aussehen von jungem Fettgewebe bekommen

Geplante Untersuchung

Die Absicht des Verfassers *war mit Hilfe* moderner Methoden die bisher dem korpereigenen Fett anhangenden Nachteile zu verringern, indem der Versuch gemacht werden sollte, eine feindisperse Emulsion herzustellen Eine dickflussige feindisperse Emulsion aus körpereigenem Fett durfte – wenn man die Frage der elektrischen Ladung einstweilen offen lasst – gute Chancen haben den oben aufgestellten Forderungen zu entsprechen, d.h. sie wurde eine *reizlose langsam aber vollständig resorbierbare* und recht effektiv trennende Zwischenschicht abgeben Ausser*dem sollte* – und das ist wichtig – diese Emulsion mit dem durch zahlreiche Untersuchungen sichergestellten Adhasionen vermindernden Effekt von Glukokortikoiden (Literatur siehe Swolin, 1966) kombiniert werden Es war weiter zu erwarten dass die Kortikoidwirkung hierdurch verbessert werden wurde da die Fettemulsion eine gleichmassigere Verteilung und ein besseres Haften der applizierten Steroide bewirken durfte

In Pilotstudien wurde versucht, mit Hilfe von Ultraschall oder mechanischen Hilfsmitteln eine Emulsion aus Glukokortikoiden und körpereigenem Fett mit und ohne Beimischung von Dextranen verschiedenster Molekülgewichte und (oder) Antibioticis herzustellen – hierbei wurden Chloroform-Methanolextraktionen (Doz. Svennerholm, med.-chem. Inst. Univ. Göteborg) der totalen Lipoidfraktion aus frischem Fettgewebe von sowohl Ratten wie Menschen verwandt — gleichzeitig wurden Versuche an 50 Albinoratten ausgeführt, in denen die Auslösung von Verwachsungen und die Wirkung der verschiedenen Emulsionen studiert wurden. Eine Anwendung von den im allgemeinen heute üblichen Emulgatoren konnte leider nicht in Frage kommen, da hierdurch toxische Effekte und eine Gewebereizung bei intraperitonealer Anwendung zu befürchten waren. Die hergestellten Emulsionskombinationen erwiesen sich als wenig stabil, ausserdem verhinderte das Risiko der Oxydierung – und damit das Auftreten von toxischen und eventuell das Peritoneum reizenden Substanzen – eine Lagerung unter für die praktische Anwendung akzeptablen Bedingungen. Kurz gesagt, es erwies sich, dass die Herstellung der gewünschten, feindispersen Emulsion aus körpereigenem Fett und Glukokortikoiden, die ohne zu grosse Schwierigkeiten auch für die praktische Anwendung in der Klinik in Frage kommen könnte, mit den dem Verfasser im Augenblick zur Verfügung stehenden sowohl technischen wie auch materiellen Hilfsmitteln nicht möglich war. Daher wurde der Versuch unternommen, eine auf dem Markt befindliche zur intravenösen Anwendung bestimmte Fettemulsion mit einem Cortisonderivat zu mischen. In Zusammenarbeit mit der Arzneimittelfirma Vitrum AB, Stockholm, wurden Emulsionen von Prednisolon und Lipid hergestellt, die dann im Tierversuch auf ihren adhäsionsvermindernden Effekt geprüft wurden.

Eigene Versuche

Versuchssubstanzen

Zu Beginn der folgenden Versuche zu Anfang des Jahres 1964 stand eine 20 %ige Lipidemulsion, das Intralipid (Oleum sojae fractionat. 20 g, Lecithinum fractionat. e vitello ovi 1,2 g, Glycerol

2 5 g aqua steril. ad 100 ml) der Arzneimittelfirma Vitrum AB Stockholm, mit und ohne Zusatz von 2 5 % Prednisolon (WHO) zur Verfugung. Das Intralipid ist eine Fettemulsion zur intra venösen Infusion. Später stand eine Emulsion vom Typ Intralipid 30 %ig mit 2 5 % Prednisolon zur Verfügung Da diese 30 %ige Emulsion leider die Tendenz zeigte etwas instabil zu sein, wurde zum Schluss der Versuche eine mit Hilfe von neueren technischen Verfahren hergestellte stabile Emulsion von 30 % igem Intralipid mit 0 2 % Prednisolon dem Verfasser zur Ver fügung gestellt.

Versuchstiere und allgemeine Versuchsbedingungen

Als Versuchstiere standen Albinoratten beiderlei Geschlechts mit Gewichten zwischen 200 g und 400 g zur Verfügung. Die Ratten wurden in Gruppen zu je 5 Tieren in modernen Plastikkäfigen unter gleichen diätetischen und klimatischen Bedingungen gehalten und waren dem ausseren Anschein nach frei von Krank heiten. Die Kost bestand aus einer speziell für Mause und Ratten ausgearbeiteten Standardkost die von einer Arzneimittelfirma (AB Ferrosan, Malmo) in Form von Pellets hergestellt wird. Nach Angaben der Firma enthalten diese Presslinge Weizenmehl und -kleie Gersten und Mischgetreideschrot Soja und Luzernemehl, Fleisch und Fischmehl sowie Magermilchpulver weiterhin Vita min A B_1, D_2, E Nikotylamid Calciumpantothenat, Folinsäure und Cholinbitartrat sowie als Spurenelemente Kobalt Mangan Ferro- und Magnesiumsulfat und Kochsalz. Zu trinken bekamen die Tiere Leitungswasser Nahrung und Wasser waren ad libitum. In der Regel waren die Tiere vor der Operation weder fastend noch durstend. Als Unterlage hatten die Tiere in den Plastik käfigen eine dicke Schicht von Sägespänen die erste Zeit nach der Operation lagen die Tiere auf reinem Filtrierpapier Die Ver suche wurden immer in Gruppen zu 5 Tieren durchgeführt meistens gelang es an einem Nachmittag und Abend zwei Grup pen zu operieren. Abgesehen von der obenerwähnten Pilotstudie mit körpereigenem oder humanem Fett an 50 Tieren wurden in dieser Studie in den Jahren 1964 und 1965 325 Ratten operiert.

Operationsmethodik. Adhäsionserzeugung und Reoperation

Abgesehen von drei Versuchsgruppen, bei denen eine intramuskuläre Barbituratnarkose versucht wurde wurden alle Tiere einer Äthernarkose unterworfen. Wenn auch Ratten im allgemeinen als recht unempfindlich gegenüber Infektionen gelten, so waren die geplanten Untersuchungen doch in einem hohen Grade fur Komplikationen in Form einer Wundinfektion (Thomaschek, 1959) prädestiniert, weshalb grosster Wert auf eine sorgfältige Sterilität während der Operation gelegt wurde (sterile Instrumente und Tücher Mundschutz etc.) Nach Beginn der Narkose wurde die Bauchhaut der Tiere unter Zuhilfenahme von pHisoHex® (3 %iges Hexachlorophenpräparat, Winthrop Lab New York) rasiert. Darauf wurden die Tiere mit Hilfe von vier grossen Nadeln auf einer Korkplatte fixiert, worauf die Bauchhaut mit sterilen Tupfern und etwa 80 %igem Alkohol getrocknet wurde. Dann wurden die Tiere mit sterilen Tüchern abgedeckt. Die Bauchhöhle wurde durch einen 3—4 cm langen zentralen Mittellinienschnitt geöffnet, wobei die Hautkanten durch zwei Klammern an den Schlitz des sterilen Tuches fixiert wurden. Hierdurch wurde ein Offenhalten der Wundkanten und eine zusatzliche Sicherung des Operationsfeldes gegen eine externe Infektion erreicht.

Nach einigen Vorversuchen wurde die Adhäsionsauslösung immer durch Quetschung des Caecum mit einem 5 mm breiten Pean hervorgerufen. Durch sorgfältiges Ausstreichen wurde das Caecum von allem Inhalt geleert, worauf der Darm, von dem Caecumende her beginnend, an drei Stellen mit etwa je 5 mm Zwischenraum geklemmt wurde (siehe Abbildung 1 und 2) Hierbei wurde der Péan während etwa 5 Sekunden völlig geschlossen. In Übereinstimmung mit anderen Verfassern (Chandy und Rhoads 1946 Thomas et al 1950 1951 Thomaschek, 1959 Ehlers und Gr[illegible]mschl 1960) führte die Quetschung des Caecum bei den Kontroll [illegible]suchen zu einer 100 %igen Adhasionsauslosung ([illegible]ehe Abbildung 3 und 4)

In den Fallen, in denen eine Flüssigkeit (Prednisolon, Intralipid oder eine Mischung [illegible]on beiden) in den Bauchraum appliziert wurde wurde das vorgelagerte Caecum mit einigen Tropfen

dieser Emulsion bedeckt *und vorsichtig eingeschmiert. Hierbei* lag das Caecum auf einem kleinen, sterilen Plastikstreifen (Format etwa 4×6 cm) um zu vermeiden dass die Emulsion von dem das Operationsfeld abdeckenden Tuche *absorbiert wurde*. Danach wurde das Caecum in die Bauchhohle zurückverlagert und der Rest der Flüssigkeit mit Hilfe eines dunnen Plastikkatheters unter die Bauchdecken eingefullt Hierauf wurde die Emulsion sorgfaltig und vorsichtig mit dem stumpfen Ende einer Pinzette im gesamten Bauchraum verteilt. Mit einer fortlaufenden atraumatischen Catgutnaht (0000 chromiert) wurden Peritoneum und Muskulatur geschlossen, wobei immer versucht wurde sorgfaltig alle eventuellen Reste einer eingefüllten Flüssigkeit von den Wundkanten abzutrocknen – Anzumerken ist hier dass es wichtig ist eine sehr feine atraumatische Nadel zu benutzen, damit nicht die in den Bauchraum eingefullte Flüssigkeit durch die Stichkanale in die Wunde kommt – Die Hautnaht geschah mit *Hilfe* einer fortlaufenden Naht aus geflochtenem, rostfreiem Stahl (00 000) Zum Schluss wurde ein flussiger Wundverband (Nobecutanspray® AB Bofors Bofors) auf die *Wunde aufgespruht*

Die Reoperation und Kontrolle der Tiere geschah am 10 Tage post operationem Zu diesem Zeitpunkt ist die Adhäsionsbildung *als abgeschlossen zu betrachten* (Graser 1888 Hinsberg 1900 Roloff 1900 Thomas und Rhoads 1950 Williams 1954, 1955 Thomaschek 1959 Ehlers und Grimsehl 1960 Ellis 1962 Kousaki 1963) Es erschien deshalb unnotig, die Beobachtungszeit uber einen langeren Zeitraum auszudehnen

Im Laufe der Untersuchungen wurde ein System zur exakten Kontrolle und Beschreibung der Verwachsungen bei der Reoperation ausgearbeitet. Folgende Bezeichnungen wurden angewendet I) Verwachsung vom Caecum ausgehend II) Verw von anderen Organen oder Därmen ausgehend III) Verw von der Bauchwand ausgehend. Breite der Verw a) 0–2 mm b) 2–10 mm c) mehr als 10 mm d) Konglomerattumor Anzahl der Verw 1 *oder 2 usw Löslichkeit der Verw* α leicht löslich β ziemlich leicht löslich, γ schwer löslich Dicke der Verw $\not\prec$ dunne Verw $\neq$ mitteldicke Verw $\not\leq$ kraftige Verw

dieser Emulsion bedeckt und vorsichtig eingeschmiert. Hierbei lag das Caecum auf einem kleinen sterilen Plastikstreifen (Format etwa 4×6 cm) um zu vermeiden, dass die Emulsion von dem das Operationsfeld abdeckenden Tuche absorbiert wurde. Danach wurde das Caecum in die Bauchhohle zurückverlagert und der Rest der Flüssigkeit mit Hilfe eines dunnen Plastikkatheters unter die Bauchdecken eingefullt Hierauf wurde die Emulsion sorgfältig und vorsichtig mit dem stumpfen Ende einer Pinzette im gesamten Bauchraum verteilt. Mit einer fortlaufenden atraumatischen Catgutnaht (0000 chromiert) wurden Peritoneum und Muskulatur geschlossen, wobei immer versucht wurde sorgfaltig alle eventuellen Reste einer eingefüllten Flüssigkeit von den Wundkanten abzutrocknen. – Anzumerken ist hier dass es wichtig ist eine sehr feine atraumatische Nadel zu benutzen damit nicht die in den Bauchraum eingefüllte Flüssigkeit durch die Stichkanäle in die Wunde kommt. – Die Hautnaht geschah mit Hilfe einer fortlaufenden Naht aus geflochtenem, rostfreiem Stahl (00 000) Zum Schluss wurde ein flussiger Wundverband (Nobecutanspray® AB Bofors Bofors) auf die Wunde aufgespruht.

Die Reoperation und Kontrolle der Tiere geschah am 10 Tage post operationem Zu diesem Zeitpunkt ist die Adhasionsbildung als abgeschlossen zu betrachten (Graser 1888 Hinsberg, 1900 Roloff 1900 Thomas und Rhoads 1950 Williams, 1954 1955 Thomaschek 1959 Ehlers und Grimschl, 1960 Ellis 1962 Kousaki 1963) Es erschien deshalb unnotig die Beobachtungszeit uber einen längeren Zeitraum auszudehnen

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dieser Emulsion bedeckt und vorsichtig eingeschmiert. Hierbei lag das Caecum auf einem kleinen, sterilen Plastikstreifen (Format etwa 4×6 cm), um zu vermeiden, dass die Emulsion von dem das Operationsfeld abdeckenden Tuche absorbiert wurde. Danach wurde das Caecum in die Bauchhohle zurückverlagert und der Rest der Flussigkeit mit Hilfe eines dünnen Plastikkatheters unter die Bauchdecken eingefullt. Hierauf wurde die Emulsion sorgfältig und vorsichtig mit dem stumpfen Ende einer Pinzette im gesamten Bauchraum verteilt. Mit einer fortlaufenden, *atraumatischen Catgutnaht (oooo chromiert) wurden Peri*toneum und Muskulatur geschlossen, wobei immer versucht wurde, sorgfaltig alle eventuellen Reste einer eingefullten Flüssigkeit von den Wundkanten abzutrocknen. – Anzumerken ist hier, dass es wichtig ist, eine sehr feine atraumatische Nadel zu benutzen, damit nicht die in den Bauchraum eingefullte Flussigkeit durch die Stichkanäle in die Wunde kommt. – Die Hautnaht geschah mit Hilfe einer fortlaufenden Naht aus geflochtenem, rostfreiem Stahl (oo ooo). Zum Schluss wurde ein flüssiger Wundverband (Nobecutanspray® AB Bofors, Bofors) auf die Wunde aufgesprüht.

Die Reoperation und Kontrolle der Tiere geschah am 10. Tage post operationem. Zu diesem Zeitpunkt ist die Adhäsionsbildung als abgeschlossen zu betrachten (Graser 1888, Hinsberg 1900, Roloff 1900, Thomas und Rhoads 1950, Williams 1954, 1955, Thomaschek 1959, *Ehlers und Grimsehl* 1960, Ellis 1962, Kousaki 1963). Es erschien deshalb unnotig, die Beobachtungszeit uber einen langeren Zeitraum auszudehnen.

Im Laufe der Untersuchungen wurde ein System zur exakten Kontrolle und Beschreibung der Verwachsungen bei der Reoperation ausgearbeitet. Folgende Bezeichnungen wurden angewendet: I) Verwachsung vom Caecum ausgehend, II) Verw. von anderen Organen oder Darmen ausgehend, III) Verw. von der Bauchwand ausgehend. Breite der Verw.: a) 0–2 mm, b) 2–10 mm, c) mehr als 10 mm, d) Konglomerattumor. Anzahl der Verw.: 1 oder 2 usw. Loslichkeit der Verw.: α leicht löslich, β ziemlich leicht löslich, γ schwer loslich. Dicke der Verw.: $\rightharpoonup$ dünne Verw., $\neq$ mitteldicke Verw., $\nLeftarrow$ kraftige Verw.



Abbildung 3 Durch Caecumquetschung ausgelöste Adhäsionen zwischen Caecum, Omentum und Dünndarm. (Beachte die Schrumpfung des distalen Caecum und die Klemmenspuren)



Abbildung 4 Durch Caecumquetschung ausgelöste Adhäsionen zwischen dem geschrumpften, distalen Teil (↓) des Caecum auf der einen Seite und dem dickeren proximalen Teil des Caecum, dem Dünndarm und dem Omentum auf der anderen Seite. (Beachte die fast völlige Einhüllung des gequetschten Caecumteils mit Omentum und Bindegewebe)



Abbildung 1 Auslösung von Adhäsionen mit Hilfe der Quetschung des Caecum. *(Beachte die Klemmspuren mit der fast augenblicklichen* Exsudation von Blut.)



Abbildung 2: Auslösung von Adhäsionen mit Hilfe der Quetschung des Caecum. (Beachte Péantyp und die durch ihn verursachte fast durchsichtige Quetschung des Caecum.)

Bei keinem der hier in dieser Arbeit beschriebenen Versuche wurde eine Infektionsprophylaxe mit Antibioticis durchgeführt. Hierdurch wurde vermieden, dass ein weiterer Faktor in die Untersuchungen eingeführt wurde, dem – vor allem bei der gewählten Technik der Adhäsionserzeugung – ein Einfluss auf die Bildung von postoperativen Verwachsungen hätte zugeschrieben werden können.

Einteilung und Übersicht der Versuchsserien

Die Versuche mit einer Emulsion von Lipid und Kortikosteroid können der Übersichtlichkeit halber zu neun Versuchsserien zusammengefasst werden. Versuchsserie I und II sind gewisser massen als Vorversuche zu betrachten.

In *Serie I* (50 Ratten) sollte unter verschiedenen Versuchsbedingungen ein erster Überblick über ein Kombinationspräparat (20 %iges Intralipid mit einem Gehalt von 2,5 % Prednisolon) gewonnen und eine standardisierte Operationsmethodik herausgearbeitet werden. Variert wurden in dieser Serie die Menge der applizierten Emulsion (0 5–10 ml) der Tag der Reoperation, die Narkose, die Art des Wundverschlusses

In Serie II (35 Ratten) wurde die Art und Weise der Adhäsionserzeugung gewechselt. Verwachsungen wurden hervorgerufen durch Quetschung des Caecum mit verschiedenen Péantypen, Reiben des Caecum mit trockenen Tüchern, Betupfen des Caecum mit 96 %igem Alkohol und Quetschen des Caecum mit nachfolgendem Betupfen mit konzentriertem Alkohol.

Serie III (50 Ratten) enthält die Kontrolltiere Das heisst die Tiere wurden einer standardisierten Caecumquetschung (siehe oben unter Operationsmethodik) unterworfen, um zu sehen, in welchem Ausmass mit dieser Methode Adhäsionen ausgelöst werden konnten

Versuche in denen nur Lipid (20 %iges Intralipid) appliziert wurde finden sich in Serie IV (35 Ratten) Es wurden die Resorptionsgeschwindigkeit, der Zustand der Ratten zwei Monate nach der Applikation und die Beeinflussung von Verwachsungen studiert In der ersten Gruppe dieser Serie wurden fünf Tieren 2 ml Lipid nach Öffnung der Bauchhöhle instilliert. Die Ratten sollten eigentlich darauf mit eintägigem Intervall reoperiert wer

Serie I Agraffen und Seidennähte erwiesen sich als unzweckmässig für den Schluss der Bauchhaut. Die Naht der inneren Bauchwunde (Peritoneum und Muskulatur) musste mit sehr feinen, atraumatischen Nadeln geschehen, da sonst zu leicht Flüssigkeit aus der Bauchhöhle durch die Stichkanäle zwischen die Bauchdecken gelangen konnte. 2 ml Flüssigkeit schien in etwa die grösste Menge zu sein, die ohne grössere Schwierigkeiten zu applizieren war und die nicht von zu vielen Komplikationen gefolgt war Bei einer Instillation von 2 ml Lipidkombination konnte man noch am vierten Tage post operationem eine hauchartige fettschimmernde Schicht auf der Leberoberfläche feststellen, bei Applikation von nur einem Milliliter fand sich bereits nach zwei Tagen keine Spur mehr

Serie II Die Quetschung des Caecum mit einem recht kräftigen, etwa 5 mm breiten Péan (siehe Abbildung 1 und 2) erwies sich als die sicherste und dabei objektivste Methode der Adhäsionserzeugung.

Serie III Kein Tier dieser Gruppe, die mit ihren 50 Ratten das Kontrollmaterial bildet war ohne Adhäsionen (siehe Abbildung 3 und 4) (Die Verwachsungen liessen sich als schwere – in der Mehrzahl der Fälle – oder als mittelschwere einstufen.] Die Sicherheit der Adhäsionsauslösung war also als 100 %ig zu betrachten. Diese Befunde entsprechen den Erfahrungen anderer Autoren (Chandy und Rhoads, 1946 Thomas *et al.* 1950 1951 Thomaschek, 1959 Ehlers und Grimsehl, 1960) Folgende Komplikationen fanden sich 1 Tier war moribund am 5. Tage (Tötung mit Äther Abszess an der Caecumspitze und zahlreiche Adhäsionen) je 2 Hämatome und 2 Serome in der Bauchwunde 1 eitrige Aussenwunde und 6 etwa bohnengrosse Wundschorfe.

Serie IV Die Tabelle IV zeigt die Ergebnisse dieser Versuche mit reinem Intralipid nach vorhergehender Quetschung des Caecum

Die Adhäsionsfreiheit der gesamten Serie IV ist 10 % [Die Verwachsungen konnten als sehr leichte oder leichte – der grösste Teil eingestuft werden.)

Bei weiteren fünf Ratten sollte die Resorption von 2 ml Intralipid durch Reoperation mit eintägigem Intervall kontrolliert wer

den, um die Resorptionsgeschwindigkeit festzustellen. Da jedoc bereits am zweiten Tage post operationem keine Lipidlösun mehr zu sehen war wurden die übrigen drei Tiere zwei Mona am Leben gelassen um eventuelle Spätfolgen einer intraperit nealen Applikation von 2 ml Intralipid zu beobachten

In *Serie V* finden sich die 50 Ratten, die mit einer intraperit nealen Applikation von 2 ml Lipid comp (20 %iges Intralipi und 2 5 %iges Prednisolon) den Kernpunkt der hier referierte Untersuchungen bilden

Serie VI umfasst die Versuche bei denen die Tiere nur Predn solon nach einer vorhergehenden Caecumquetschung erhielte 30 Tiere erhielten eine 2 5 %ige Suspension von mikrokrista linem Prednisolon in physiologischer Kochsalzlösung und 20 Tie eine 0 25 %ige Suspension

In *Serie VII* (15 Ratten) wurde der adhäsionsvermindernd Effekt einer Lipidkombination untersucht, die etwas dickflüssige war und die in 30 %igem Intralipid 2 5 % Prednisolon enthiel Diese Emulsion war durch den Zusatz des Prednisolon etwa instabil geworden so dass sich während der Lagerung ein Te des Fettes separiert hatte durch kräftiges Schütteln kurz vor de Anwendung konnte jedoch die Fettschicht – makroskopisch we nigstens – wieder zum Verschwinden gebracht werden.

Serie VIII (30 Ratten) beleuchtet das Verhalten der Ver wachsungsfrequenz nach intraperitonealer Gabe von 2 ml eine Kombination von 30 %igem Intralipid mit einem Zusatz vo 0 2 % Prednisolon bei der mit Hilfe einer verfeinerten Techni eine Stabilisierung der Emulsion gelungen war

Zum Schluss wird in *Serie IX* (10 Ratten) die Resorption und der Einfluss von 2 ml Lipid comp (20 %iges Intralipid mit 2 5 % Prednisolon) nach und ohne Caecumquetschung mit einem Inter vall von 1 2, 4 8 und 16 Stunden untersucht

Ergebnisse

Da die Untersuchungen der Serien I und II gewissermassen als Vorversuche angesehen werden können sollen nicht die Ergebnisse im Detail sondern nur die Schlussfolgerungen mitgeteilt werden, die sich aus diesen Versuchen ziehen lassen.

Serie I Agraffen und Seidennähte erwiesen sich als unzweckmässig für den Schluss der Bauchhaut. Die Naht der inneren Bauchwunde (Peritoneum und Muskulatur) musste mit sehr feinen, atraumatischen Nadeln geschehen, da sonst zu leicht Flüssigkeit aus der Bauchhöhle durch die Stichkanäle zwischen die Bauchdecken gelangen konnte 2 ml Flüssigkeit schien in etwa die grösste Menge zu sein, die ohne grössere Schwierigkeiten zu applizieren war und die nicht von zu vielen Komplikationen gefolgt war Bei einer Instillation von 2 ml Lipidkombination konnte man noch am vierten Tage post operationem eine hauchartige fettschimmernde Schicht auf der Leberoberfläche feststellen, bei Applikation von nur einem Milliliter fand sich bereits nach zwei Tagen keine Spur mehr

Serie II Die Quetschung des Caecum mit einem recht kräftigen, etwa 5 mm breiten Péan (siehe Abbildung 1 und 2) erwies sich als die sicherste und dabei objektivste Methode der Adhäsionserzeugung.

Serie III Kein Tier dieser Gruppe, die mit ihren 50 Ratten das Kontrollmaterial bildet war ohne Adhäsionen (siehe Abbildung 3 und 4) [Die Verwachsungen liessen sich als schwere – in der Mehrzahl der Fälle – oder als mittelschwere einstufen.] Die Sicherheit der Adhäsionsauslösung war also als 100 %ig zu betrachten. Diese Befunde entsprechen den Erfahrungen anderer Autoren (Chandy und Rhoads 1946 Thomas *et al.* 1950 1951 Thomaschek, 1959 Ehlers und Grimsehl, 1960) Folgende Komplikationen fanden sich 1 Tier war moribund am 5. Tage (Tötung mit Äther Abszess an der Caecumspitze und zahlreiche Adhäsionen) je 2 Hämatome und 2 Serome in der Bauchwunde 1 eitrige Aussenwunde und 6 etwa bohnengrosse Wundschorfe

Serie IV Die Tabelle IV zeigt die Ergebnisse dieser Versuche mit reinem Intralipid nach vorhergehender Quetschung des Caecum.

Die Adhäsionsfreiheit der gesamten Serie IV ist 10 % [Die Verwachsungen konnten als sehr leichte oder leichte – der grösste Teil eingestuft werden.]

Bei weiteren fünf Ratten sollte die Resorption on 2 ml Intralipid durch Reoperation mit eintägigem Intervall kontrolliert wer

Tabelle IV (Serie IV) *Adhäsionen nach Caecumquetschung mit nachfolgender Applikation von Intralipid 20 %*

Anzahl Tiere	Sexus	ml Lipid	Keine Adhäsionen	Bemerkungen und Komplikationen
5	♂	1	–	5 leicht lösliche Adh
5	♂	1	1	4 leicht lösliche Adh
5	♂	1	–	4 leicht lösliche + 1 recht leicht lösliche Adh
1	♀	1	–	1 recht leicht lösliche Adh.
4	♀	2	–	4 leicht lösliche Adh
5	♂	2	1	3 leicht + 1 recht leicht lösliche Adh
5	♀	2	1	3 leicht + 1 recht leicht lösliche Adh
30	–	–	3	Keine Wundkomplikationen (23 leichte + 4 recht leicht lösliche Adhäsionen)

den Am ersten Tage post operationem fand sich noch ein zarter Fettschimmer auf den Eingeweiden. Da die Resorption makroskopisch bereits am zweiten Tage vollständig war wurden die übrigen drei Tiere zwei Monate lang am Leben gelassen, um eventuelle Spätfolgen einer intraperitonealen Lipidapplikation beobachten zu können Die Tiere zeigten äusserlich keine Minderung des Wohlbefindens und bei der Kontrollobduktion keine makroskopisch wahrnehmbaren Veränderungen Ein Tier gebar in der Zwischenzeit 11 lebende Junge Ausserdem kann man zu dieser Serie drei Tiere einer Gruppe rechnen, die 10 Tage vorher mit einer Caecumquetschung behandelt worden war und bei der der Effekt des Intralipid auf die Neubildung von gelösten Verwachsungen geprüft werden sollte Zwei von den fünf Tieren starben leider unter der Narkoseeinleitung für die Reoperation und Lipidinstillation. Die wiedergebildeten Verwachsungen der drei übrigen Tiere waren geringer deutlich dünner und leichter zu lösen.

Serie V In Tabelle V sind die Ergebnisse der Hauptversuche dargestellt.

Es bestand eine Adhäsionsfreiheit in 88 Diese Prozentzahl hat ein 95 %iges Konfidenzintervall (79 % 97 %) und zeigt somit eine klare statistisch signifikante Differenz gegenüber dem Kontrollmaterial. (Die wenigen gefundenen Verwachsungen

Tabelle V (Serie V) *Adhäsionen nach Caecumquetschung mit nachfolgender Applikation von ml Lipidgemisch (Intralipid 20 % + 2,5 % Prednisolon)*

Nr. Gruppe	Anzahl Tiere	Sexus	Keine Adhäsionen	Bemerkungen und Komplikationen
36	5	♀	5	kleines Hämatom, 1 kleine Hernie in Innenwunde
39	5	♀	4	exitus (9. Tag, angefressen, 0 Adh.) Wundabszess Serom, 2 kleine Hernien, minimale Adh.
40	5	♂	4	1 minimale Hernie in Innenwunde, minimale Adh.
41	5	♂	4	exitus (9 Tag, fast aufgefressen rotes Fleisch) kleines Hämatom, Wundschorf
43	5	♂	4	exitus (8. Tag, nur Haut übrig) kleine Hernie in Innenwunde
44	5	♀	4	kleines Serom, minimale Adh.
59	5	♂	5	
60	5	♂	5	gut linsengroßer Wundschorf
6	5	♀	4	exitus (3. Tag, Eingeweide aufgefressen blutig und rotes Fleisch 0 Leichenstarre
62	5	♀	5	
	50		44	4 exitus (ohne Adh.) 1 Wundabszess Wundschorf 5 Hernien 2 Serome 3 minimale Adhäsionen

konnten als leicht oder sehr leicht eingestuft werden.) Zu den Nebenwirkungen wird in der Diskussion der Ergebnisse Stellung genommen.

Serie VI In Serie VI wurden zum Vergleich Versuche mit der einen Komponente der Lipidkombination, nämlich dem Prednisolon, durchgeführt die Ergebnisse werden in der Tabelle VI zusammengefasst.

Die 2 5 -Gruppen hatten in 12 Fällen keine Verwachsungen, das bedeutet eine Adhäsionsfreiheit in 40 % die entsprechende Zahl für die 0 25 % -Gruppen war 45 % (Die vorhandenen Adhäsionen konnten als leichte oder mittelschwere eingestuft werden)

Serie VII In Serie VII wurde der Versuch gemacht, die Emul

Tabelle VI (Serie VI) *Adhäsionen nach Caecumquetschung und nachfolgender Prednisolonapplikation* (a. 2 ml 2,5 % b. 2 ml 0,25 %)

	Anzahl Tiere	Sexus	%	Keine Adhäsionen	Bemerkungen und Komplikationen
a	5	♀	2,5	2	1 Serom
	5	o	2,5	4	–
	5	♀	2,5	1	1 kleine Hernie 1 Tier 4.–6. Tag krank (krankes Tier 0 Adh.)
	5	♂	2,5	1	1 kleiner Wundabszess 1 grosse und 2 kleine Hernien
	5	♀	2,5	2	1 exitus (4. Tag, diffuse Peritonitis)
	5	♀	2,5	2	–
	30	–	–	12	1 exitus 1 grosse und 3 kleine Hernien 1 Serom 1 kleiner Wundabszess
b	5	o	0,25	0	2 exitus (2., 3. Tag, bilaterale Pneumonie) 1 kleiner Abszess an Caecumspitze
	5	♀	0,25	2	2 exitus (2. 3. Tag bilaterale Pneumonie) 1 Tier 3.–6. Tag schwerkrank, hustend 1 kleiner Wundschorf
	5	♂	0,25	5	–
	5	♂	0,25	2	–
	20	–	–	9	4 exitus kleiner Abszess an Caecumspitze 1 kleiner Wundschorf

sion durch Erhöhung des Lipidgehaltes weniger dünnflüssig zu machen. Ausser einem Tiere der mittleren Gruppe das 5 ml erhielt – und keine Verwachsungen zeigte – erhielten alle Tiere eine Instillation von 2 ml einer nicht völlig stabilen Lipidkombination (siehe Tabelle VII)

Es fand sich also in Serie VII eine Adhäsionsfreiheit in 47 % und eine nicht geringe Anzahl Komplikationen. (Die Verwachsungen konnten in drei Fällen als schwere und in zwei Fällen als leichte eingestuft werden.)

Serie VIII Die Tabelle VIII gibt die Resultate der Serie VIII

Tabelle VII (Serie VII). *Adhäsionen nach Caecumquetschung und nachfolgender Applikation von 2 ml Lipidgemisch* (30 %iges Intralipid + 2,5 % Prednisolon)

Anzahl Tiere	Sexus	Keine Adhäsionen	Bemerkungen und Komplikationen
5	♀	1	3 exitus (2., 3., 4. Tag, 2 Eingeweide aufgefressen, diffuse Peritonitis) grosse Hernie
5	♀	4	kleiner Abszess an Caecumspitze 1 Tier 5 ml (0 Adh.)
5	♂		kleiner Wundabszess grosse Hernie
5		7	3 exitus grosse Hernien, kleiner Abszess an Caecumspitze 1 kleiner Wundabszess

Tabelle VIII (Serie VIII) *Adhäsionen nach Caecumquetschung und nachfolgender Applikation von ml Lipidgemisch* (30 %iges Intralipid + ,2 % Prednisolon)

Anzahl Tiere	Sexus	Keine Adhäsionen	Bemerkungen und Komplikationen
5	♂		
5	♀		
5	♀	3	
5	♀	0	
5	♀		
5	♂	3	
30			Keine Komplikationen

wieder in der eine verbesserte stabile Lipidkombination mit einem geringeren Prednisolongehalt zur Verfügung stand.

Die Adhäsionsfreiheit der Serie VIII war 33 % (Alle Verwachsungen konnten als sehr leichte – die meisten – oder leichte eingestuft werden.)

Serie IX. Die Ergebnisse der Versuche in dieser Serie mit kurzen Reoperationsintervallen (1–16 Stunden) können wie folgt zusammengefasst werden. Die Wunden waren reingeleckt nach 8 Stunden, zu welchem Zeitpunkt die Tiere auch wieder ein

normales Gebaren an den Tag legten. Es fand sich kein Unterschied in dem Verhalten der Tiere mit oder ohne Caecumquetschung In allen Fallen das heisst auch bereits nach einer Stunde sah man an der inneren Seite der Bauchwunde und den Klemmstellen des Caecum zahlreiche recht weiche und nicht sehr fest sitzende weissliche, etwa grießkornchengrosse teilweise sehr dichte Ablagerungen. Ahnliche jedoch teilweise bis etwa stecknadelkopfgrosse Zusammenballungen fanden sich im Oment und vereinzelt in der gesamten Bauchhohle. Vor allem in den ersten Stunden fanden sich diese Ansammlungen im Oment, das sich gleichzeitig etwas einrollte spater war es etwas verdickt und nahm eine rosa gelbliche Farbe an wahrend es zum Schluss d.h. nach 16 Stunden, wieder eine normale Farbe hatte Kein Tier hatte Verwachsungen. Eine feinlobuläre Zeichnung der Leber fand sich bei drei Tieren (4 8 16 Stunden) mit und bei einem Tier (16 Stunden) ohne Caecumquetschung eine recht helle Farbe hatte die Leber bei drei Tieren (8 und 16 Stunden mit und 16 Stunden ohne Caecumquetschung)

Eine gewisse Resorption glaubte man bereits nach zwei Stunden feststellen zu konnen Nach vier Stunden hatte das Tier ohne Caecumschaden bereits fast alle Flüssigkeit resorbiert so dass nur noch ein dunner fettschimmernder Belag auf den Eingeweiden zu sehen war das entsprechende Tier mit dem geklemmten Colon zeigte dagegen noch reichliche Mengen der Emulsion. Nach 8 Stunden hatte das Tier ohne (sic!) Caecumquetschung noch milchähnliche Emulsionsspuren zwischen den Därmen das andere Tier hatte nur einen dunnen, fettschimmernden Belag auf den Eingeweiden. Nach 16 Stunden zeigte nur die Ratte mit dem Colonschaden einen hauchdunnen, fettschimmernden Belag uber all in der Bauchhohle wahrend man diesen bei dem anderen Tier nur auf der Leber wahrnahm

Diskussion

Solange man nicht eine Substanz finden kann, die selektiv an den geschädigten Stellen des Peritoneums haftet oder die einmal auf eine gewisse Stelle appliziert an dem gewünschten Platze liegen bleibt, ist es sicherlich von Vorteil eine moglichst grosse Flüssig

keitsmenge zu instillieren (Kern und Kuhbler 1964) Hierfür sprechen die eigenen Ergebnisse der Serien I und II und die Untersuchungen von Hertzler (1935) Es ist eigentlich erstaunlich, dass einige Verfasser mit so geringen, die aktive Substanz enthaltenden Volumina so gute Erfolge erzielen konnten, wenn man selbst gesehen hat, wie geringe Mengen von applizierter Flüssigkeit sich schnell über die gesamte Bauchhöhle verteilen und vor allem sich an den jeweils niedrigsten Punkten ansammeln, ohne an den gewünschten Plätzen liegen zu bleiben. Eine gute Illustration hierzu liefern die Untersuchungen von Steinberg (1944) aus denen zu ersehen ist, dass selbst ein öliges Medium sich schnell im Bauchraum verteilt. Die gegenüber einer wässerigen Prednisolonlösung mit einer öligen Applikationsform erzielten besseren Resultate (Solero 1958) weisen vielleicht auch in die gleiche Richtung. Aufgrund der eigenen Versuche scheinen 2 ml ein recht optimales Volumen für die Ratte zu sein.

Zur Auslösung von Verwachsungen sind viele Methoden beschrieben worden die Traumata können entweder mechanischer bakterieller chemischer oder physikalischer Art sein. Einige Beispiele sind Austrocknung (Walthard, 1893) Jodtinktur (Pribram, 1914) Skarifizierung mit dem Skalpell (Löhnberg, 1922) NaOH Applikation (Thomaschek 1959) Ischämie (Ellis, 1962) Faeceslösung (Laktionov 1962) Reiben mit trockener Gaze (Close et al. 1963) Ultraviolettstrahlung (Beehler und Clay 1965) Sandpapier (Jewett, 1965) Die von vielen Autoren zur Adhäsionserzeugung angewandte Talkumapplikation ist eine inadäquate nicht mit einem Operationstrauma zu vergleichende Methode, da hierdurch ein die Versuche weit überdauernder Reiz gesetzt wird. Die „physiologischste" Methode zur Erprobung von adhäsionsverhindernden Medien ist eigentlich die Lösung von – in einer früheren Operation erzeugten Verwachsungen. Hierfür werden jedoch grössere Tiere benötigt, die leider aufgrund von technischen Schwierigkeiten nicht zur Verfügung standen. Es ist deshalb in unseren Versuchen die Quetschung des Caecum zur Adhäsionsauslösung angewandt worden, da sich diese Methode gut standardisieren lässt und in etwa einem recht groben Operationstrauma ähnelt.

Es besteht kein Zweifel dass es Kortikosteroide gibt, die eine

normales Gebaren an den Tag legten. Es fand sich kein Unter schied in dem Verhalten der Tiere mit oder ohne Caecumquet schung In allen Fällen, das heisst auch bereits nach einer Stunde sah man an der inneren Seite der Bauchwunde und den Klemm-stellen des Caecum zahlreiche recht weiche und nicht sehr fest sitzende weissliche etwa grießkornchengrosse teilweise sehr dichte Ablagerungen. Ähnliche jedoch teilweise bis etwa steck nadelkopfgrosse Zusammenballungen fanden sich im Oment und vereinzelt in der gesamten Bauchhöhle Vor allem in den ersten Stunden fanden sich diese Ansammlungen im Oment, das sich gleichzeitig etwas einrollte spater war es etwas verdickt und nahm eine rosa gelbliche Farbe an wahrend es zum Schluss d.h. nach 16 Stunden, wieder eine normale Farbe hatte Kein Tier hatte Verwachsungen. Eine feinlobuläre Zeichnung der Leber fand sich bei drei Tieren (4 8 16 Stunden) mit und bei einem Tier (16 Stunden) ohne Caecumquetschung eine recht helle Farbe hatte die Leber bei drei Tieren (8 und 16 Stunden mit und 16 Stunden ohne Caecumquetschung)

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Diskussion

Solange man nicht eine Substanz finden kann die selektiv an den geschadigten Stellen des Peritoneums haftet oder die einmal auf eine gewisse Stelle appliziert, an dem gewünschten Platze liegen bleibt ist es sicherlich von Vorteil eine möglichst grosse Flüssig

nach der Serie VI und VII operiert worden ist. Den Grossteil der Todesfälle vor allem in Serie V möchte der Verfasser durch eine Insuffizienz der Bauchnaht erklären da die Tiere ohne vorhergehende Krankheitszeichen plötzlich an- oder aufgefressen waren. – Mehrere Tierleichen wurden noch blutig und mit hellrotem Fleisch vorgefunden. – Es ist zu erwarten, um nicht zu sagen selbstverständlich, dass sich diese Komplikationen leichter bei grösseren Tieren oder an humanem Material vermeiden lassen, da hier leichter eine korrekte Naht und vor allem von Lipid und (-oder) Kortikoid reine Wundkanten zu erhalten sind.

Da eine erst beabsichtigte Auswertung der Stärke und Ausdehnung der Verwachsungen mit Hilfe des oben angegebenen Schemas bedeutende statistische Schwierigkeiten gemacht hätte und eine objekti e Beurteilung der Intensität von Verwachsungen bei kleinen Tieren immer sehr schwierig, wenn nicht sogar un möglich ist wird nur die Adhäsionsfreiheit als Beweismaterial angeführt. Die in Parenthesen gegebenen zusammenfassenden Urteile über die Art der Adhäsionen sind lediglich als Parenthesen aufzufassen.

Das Geschlecht der Versuchstiere scheint ohne Bedeutung für die Resultate der Untersuchungen zu sein. Das Verhältnis weiblich zu männlich ist in den Versuchen der Serien III–IX 120 120

Die Tatsache dass sich kleine Zusammenballungen der applizierten Emulsion an den Wundflächen sammeln – und das bereits nach einer Stunde (siehe Serie IX) – ist sicher nicht schädlich für den Effekt der Lipid Steroidkombination Im Gegenteil! Vielleicht hat sich durch einen glücklichen Zufall eine positive Ladung der oder gewisser Teile der – Emulsion ergeben, die ihrerseits zu einem Belag der elektrisch negati en Wundflächen der Bauchhöhle (Cantacuz ne und Soru 1931 Steinberg, 1944) mit Partikeln der Emulsion führt und hierdurch ausser dem lokali ierten Steroideffekt zu einem effektiven, höchst erwünschten Auseinanderhalten der Wundflächen beiträgt Reste solcher Zusammenballungen finden sich bei einigen Tieren in Form von etwa grieß- bis reiskorngrossen weichen und aller Wahrscheinlichkeit nach völlig unschädlichen Ablagerungen, die man entweder frei und lose liegend oder leicht festsitzend an den er schiedensten Stellen der Bauchhöhle finden kann.

grössere „antiinflammatorische" Potenz als das Prednisolon be sitzen (Desaulles und Meier 1957 Diraimondo und Forsham 1958 Beickert 1964) und somit besser für die Adhäsionsprophylaxe geeignet waren. Die Löslichkeit und damit die Resorptionsgeschwindigkeit spielt sicher auch eine Rolle für die Eignung eines Kortikosteroidpräparates (Eskeland 1963) Weiterhin ist besonders im Falle der Anwendung einer Lipid emulsion die Fettlöslichkeit des Steroidpräparates ein wichtiger Faktor Schliesslich ist das elektrische Potential der Wundflächen (Cantacuzene und Soru 1931 Steinberg 1944) sicherlich ein nicht zu vernachlässigendes Detail bei den Versuchen, das Kortikosteroid an der Wundfläche zum Haften zu bringen. Aus technischen und organisatorischen Gründen war der Verfasser jedoch bei seinen Versuchen an das Prednisolon gebunden.

Die sogenannten „antiinflammatorischen Eigenschaften des Hydrocortison und des Prednisolon verhalten sich etwa wie 1 3 (Diraimondo und Forsham 1958 Beickert, 1964) Da man bei Verwendung eines Gemisches aus einer Lipidemulsion und Prednisolon mit einer gewissen Abkapselung und somit einem erheblichen Verlust der Effektivität des Steroids rechnen muss wurde von Anfang an – im Vergleich mit Thomascheks (1959) Hydrocortisondosen – ein hoher Anteil Prednisolon der Lipidemulsion zugesetzt Ein Blick auf die Ergebnisse von Serie V und VIII zeigt dass diese Mutmassung nicht ganz unberechtigt war

Im Gegensatz zu fast allen anderen Autoren die bei ihren Versuchen mit Cortisonderivaten eine Infektionsprophylaxe mit Antibioticis betreiben ist bei den eigenen Versuchen von einer Antibioticaanwendung Abstand genommen worden, um die Mög lichkeit einer Beeinflussung von Verwachsungen durch das Anti bioticum (Thomas *et al* 1950 1951) auszuschliessen.

Abgesehen von den Vorversuchen (Serie I und II) treten Kom plikationen nur in den Serien V VI VII und in der Kontroll gruppe auf Die gegenüber der Kontrollgruppe recht hohe Zahl der Komplikationen in der Hauptgruppe lässt sich mit grosser Wahrscheinlichkeit mehr oder weniger durch eine verbesserte Operationstechnik vermeiden Ein Beweis hierfür ist die Kompli kationsarmut der zweiten Hälfte der Serie V die erst später d.h

tionsprophylaxe mit Antibioticis durchgeführt wurde. Das Standardtrauma, eine Quetschung des Caecum mit einem Péan, führt bei den Kontrolltieren zu einer 100 %igen Verwachsungsauslösung.

Der Medizinischen Fakultät der Universität und der Ärztegesellschaft von Göteborg danke ich für die finanzielle Unterstützung. Meinem Chef Professor Sam Brody sowie Dozent Lars Svennerholm von dem medizinisch-chemischen Institute der Universität Göteborg und Apotheker Bengt Ajaxson von der Vitrum AB in Stockholm bin ich für alle Hilfe und stimulierende Kritik zu tiefem Dank verbunden. Statistische Probleme bearbeitete liebenswürdigerweise Dozent Uno Zackrisson von dem statistischen Institute der Universität Göteborg. Der Arzneimittelfirma Vitrum AB in Stockholm bin ich für die freundliche Überlassung der Versuchspräparate zu grossem Dank verpflichtet.

SUMMARY

A survey has been given of the problems of postoperative adhesions and of their prevention. The author's experiments (carried out on 375 albino rats) show that one intraperitoneal application of a lipid emulsion (Intralipid, Vitrum AB, Stockholm) and of prednisolone (WHO) is able to prevent adhesions in 88 % of the animals. The application was given after standardized trauma; no infection prophylaxis with antibiotics has been performed. The standardized trauma consisted in crushing an area of the caecum with a hemostat. Standardized trauma caused adhesions in all of the control animals.

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Aufgabe zukünftiger Untersuchungen wird es sein, den Versuch zu machen, festzustellen, welcher Anteil des Intralipid für den adhasionsvermindernden Effekt verantwortlich ist ob Teile der *Lipid Steroidkombination* eine positive elektrische Ladung haben oder erhalten können und ob es möglich ist, die Konsistenz der Emulsion dickflüssiger zu machen. Hierdurch wird es dann vielleicht möglich sein, ein noch besseres Praparat für die Adhäsionsprophylaxe herzustellen. Weiter ware es wunschenswert und interessant, die Wirkung des Praparates an anderen (grösseren) Tieren und da vor allem seinen Einfluss auf die Wiederbildung von vorher gelosten Verwachsungen zu studieren.

Konklusion

Aus den oben angeführten Untersuchungen lassen sich – für die Ratte – folgende Schlusse ziehen Die Resorption einer in die Bauchhöhle applizierten Lipidemulsion scheint langsamer vor sich zu gehen je grosser die applizierte Menge ist weiter scheint die Resorption sowohl durch eine Quetschung des Caecum wie auch durch eine Zugabe von Prednisolon zum Intralipid verzögert zu werden. Kurze Zeit nach der Operation zeigt sich eine bevorzugte Ansammlung von Partikeln einer Intralipid Prednisolonemulsion an den traumatisierten Peritonealflächen Eine intraperitoneale Glukokortikoidapplikation zur Vermeidung von Adhasionen ist auch ohne eine gleichzeitige Infektionsprophylaxe mit Antibioticis möglich Intralipid und Prednisolon wirken in einer Kombination synergistisch und verhindern in 88 % der Fälle das Auftreten von Adhäsionen nach einem standardisierten Trauma das bei Kontrolltieren zu einer 100 %igen Verwachsungsauslösung fuhrt.

Zusammenfassung

Es wird eine Übersicht zum Problem der postoperativen Verwachsungen und ihrer Verhütung gegeben In eigenen Versuchen mit 375 Albinoratten wird gezeigt, dass eine einmalige intraperitoneale Applikation einer Emulsion aus Intralipid und Prednisolon nach einem standardisierten Trauma zu einer Adhasionsfreiheit in 88 % der Fälle führt, ohne dass hierbei eine Infek

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From the Clinic of Obstetrics and Gynecology (Professor Carl Gemzell)
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AN APPRAISAL OF THE CLINICAL EFFECT OF THREE DIFFERENT ORAL CONTRACEPTIVE AGENTS AND THEIR INFLUENCE ON TRANSAMINASE ACTIVITY

BY

ULF LARSSON-COHN

The number of reports on the clinical use of oral contraceptives is increasing rapidly It is striking that authors describe very different side reaction rates with identical drugs. Differences in the psychological disposition and in the social or economic status of patients may explain this discrepancy in part. It may also be a reflection of the thoroughness with which patients are supervised, the way they are questioned and the personal attitude and the number of the investigators.

This article describes a clinical trial of 3 different oral contraceptives at the University Hospital Uppsala. The drugs were supplied to the patients free of charge and it should be noted that the patients were assessed and supervised by only one investigator

Drugs and Dosages

The drugs used were Aconcen, Volidan and Wy 3707 Each Aconcen tablet contained chlormadinone acetate 3 mg and ethinylestradiol 3-methyl ether (EE3ME) 0.1 mg. The manufacturer was E. Merck AG Darmstadt Germany Each tablet of Volidan was made up of megestrol acetate 4 mg. and ethinylestradiol (EE) 0.05 mg. It was manufactured by The British Drug Houses Ltd London England. Wy 3707 contained 13-ethyl 1 -alpha-ethynyl-hydroxygon 4-en 4-one 1 mg and EE

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ULF LARSSON-COHN

The number of reports on the clinical use of oral contraceptives is increasing rapidly It is striking that authors describe very different side reaction rates with identical drugs. Differences in the psychological disposition and in the social or economic status of patients may explain this discrepancy in part. It may also be a reflection of the thoroughness with which patients are supervised, the way they are questioned and the personal attitude and the number of the investigators.

This article describes a clinical trial of 3 different oral contraceptives at the University Hospital, Uppsala. The drugs were supplied to the patients free of charge and it should be noted that the patients were assessed and supervised by only one investigator

Drugs and Dosages

The drugs used were Aconcen Volidan and Wy 3707 Each Aconcen tablet contained chlormadinone acetate 3 mg and ethinylestradiol 3-methyl ether (EE3ME) 0.1 mg The manufacturer was E. Merck AG Darmstadt Germany Each tablet of Volidan was made up of megestrol acetate 4 mg. and ethinylestradiol (EE) 0.05 mg. It was manufactured by The British Drug Houses Ltd. London, England. Wy 3707 contained 13-ethyl 17-alpha-ethynyl hydroxygon 4-en 4-one 1 mg and EE

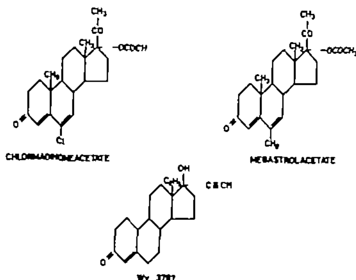


Fig 1 Chemical structures of the gestagens in the three drugs used.

0 05 mg in each tablet. The drug was made by Wyeth Laboratories Inc Radnor Pennsylvania USA

Chlormadinone acetate megastrol acetate and 13-ethyl 17-alpha-ethynyl hydroxygon 4-en 3-one are all strongly gestagenic substances with anti-oestrogenic effect. The first two derivates of acetoxyprogesterone have no androgenic effect while the third substance a 19-norsteroid derived from testosterone has an androgenic activity which is 7 5 per cent of that of testosterone

Aconcen and Wy 3707 were both given in doses of one tablet daily for the 21 days between the 5th and 25th days of the menstrual cycle The dose of Volidan was one tablet daily for the 20 days between day 5 and day 24.

Choice of Patients and Supervision

In all cases the aim of the medication was contraception. The age distribution of the patients in the trial is given in Table I 88 of the patients were married The 7 women who for different reasons transferred from one drug to another appear in the two columns relevant to each respective drug.

Table I. Number of Women Put on the Three Drugs Divided into Age Groups

Age Groups in Years	Number of Patients on		
	Aconcen	Volidan	Wy 3707
17–20	4	1	0
1–30	20	48	41
3 40	6	0	3
4 49	1		

In each case, a careful history was followed by gynæcological examination and cytological smears were taken from the cervix and the vagina. The patients were given a supply of the chosen drug in the packing provided by the manufacturer The number of patients using Aconcen was small, because this drug became available later than the other

Thorough instruction about the tablets was supplemented by a pamphlet explaining the basic principles of oral contraception. Each patient was asked to keep an accurate record of her menstrual dates and the days of therapy on a specially designed card. The patients were seen by only one doctor throughout, first after one month of treatment and then on alternate months.

With a few exceptions, blood was drawn at every visit for determination of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) The determinations were made according to Ordell s modification (1956) of the method of Karmen and Wróblewski. A reading of 40 units was considered the upper limit of normal and control determinations were made on every blood sample where one of the transaminases showed an activity of 30 units or more.

Results

The drugs were used during 1442 cycles (see Tables II and IX) No pregnancy occurred.

Table II includes 6 women who did not return to the clinic after the first visit. Two patients on Wy 3707 were admitted to the medical department with provisional diagnoses of myocardial

Table II. *Number of Cycles with Each Drug and the Number of Women Who Left the Trial Prematurely*

Drug	No. of Patients	No. of Cycles	No. of Patients Who Left the Trial: Because of Relevant Side Effects	No. of Patients Who Left the Trial: Total
Aconcen	31	217	5=16 %	10
Volidan	69	654	12=17 %	30
Wy 3707	65	571	10=15 %	23
Totals	165	1442	27=16 %	63

Table III *Analysis of Reasons for Discontinuing Medication*

Reason	Number of Patients: Aconcen	Volidan	Wy 3707
Mental depression / Irritated nervous	1	1	7
Decreased libido	1	4	1
Nausea vomiting	1	5	1
Break through bleedings	2	1	
Increased weight		1	1
Did not return	5	3	7
Planning pregnancy		4	3
Moved from area		4	1
Miscellaneous reasons		8	2

infarction and brachial vein thrombosis respectively Neither diagnosis was confirmed but both patients were advised (by their physicians) to discontinue oral contraceptives These two cases do not figure in column 4 of Table II

Table III shows the reasons for discontinuing medication

Side Reactions

At each visit the patients were asked if they had experienced any reactions since last seen and care was taken to avoid leading questions. All troubles that could reasonably be attributed to medication were noted

Table IV *Side Reactions Mentioned Spontaneously The Figures Give the Number of Patients in Each G oup*

Side Reactions	Aconcen	Volidan	Wy 3707
Nausea, vomiting	6= 1 %	28=4 %	22=38 %
Irritated mental depression, nervous	3= %	9=13 %	5=24 %
Headache		7	5
Tiredness	1	6	2
Pelvic pains		3	5
Increased appetite	3	5	
Breast tenderness	4	8	4
Vaginal discharge		3	5
Decreased libido		3	4
Miscellaneous		7	1
No troubles	9=32 %	5=22 %	6=26 %

Table V Percentage of Patient Experiencing Nausea during the First 5 Cycles

Cycle	Incidence of Nausea in Per Cent		
	Aconcen	Volidan	W 3707
		38	3
	8	14	9
3	8	1	7
4	4	3	4
5	4	3	4

The most common complaint was nausea. However it disappeared rapidly in most cases and was entirely absent at 12 months.

Weight Changes

The known tendency for oral contraceptives to cause weight increase may be due to augmented appetite water retention caused by oestrogens or the anabolic action of gestagens. Table VI shows the changes in weight observed in the patients who completed at least one month of therapy

Volidan seemed to cause less weight increase than the other drugs.

Table VI. *Changes of Weight during Therapy*

Change of Weight in Kilograms	Number of Patients		
	Aconcen	Volidan	Wy 3707
−3.0 to −4.9		1	3
−1.0 to −2.9	4	14	4
+0.9 to −0.9	7	25	13
+1.0 to +2.9	12	17	19
+3.0 to +4.9	3	5	11
+5.0 to +7.9			3
+1.0 to +7.9	15=58 %	22=35 %	37=65 %

Table VII. *Length of Cycles Expressed as Percentage of All Cycles with Each Drug*

Length of Cycle in Days	Aconcen	Volidan	Wy 3707
24		3.1	
25	2.1	9.6	2.3
26	4.1	40.5	4.3
27	23.3	38.2	23.8
28	42.5	8.2	48.2
29	18.6	0.7	18.1
30	7.8	0.5	2.9
31	1.6	0.2	0.4

Changes in Bleeding Pattern

Table VII gives the relative frequency of different cycle lengths with the three drugs. Cycles during which tablets were taken incorrect or where break through bleeding occurred were excluded as were those which were followed by amenorrhoea.

Even after allowing for the fact that it was given for one day less than the other drugs, Volidan seemed to give somewhat shorter cycles than Aconcen and Wy 3707.

Before therapy the mean duration of each menstrual period was 5.5 days in all three groups. During the trial the mean duration of bleeding was 4.8 days for Aconcen, 4.2 days for Volidan and 4.2 days for Wy 3707.

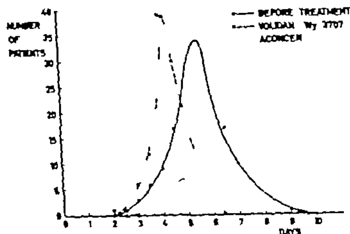


Fig. 2. Duration of bleeding befor and during treatment

Table VIII. *Subjective Estimates of the Effect of the Drugs on the Amount of Bleeding*

Patient's Estimate of Bleeding	Per Cent of Total Number of Cycles with Each Drug		
	Aconcen	Volidan	Wy 3707
Scantier	86.4	87.8	79.5
Unchanged	7	9.8	[illegible]
Heavier	9	2.4	5

Fig. 2 shows the distribution of the duration of bleeding before and during therapy. As the distribution among the women who used Volidan and Wy 3707 was practically identical, the results were combined to give one graph. There was a tendency to longer bleedings in users of Aconcen.

The patients subjective opinion about the quantity of the bleedings during therapy are given in Table VIII.

Irregularities of Bleeding

Three kinds of irregularity occur during oral contraceptive therapy: break-through bleeding (BTB), spotting and amenor

Table IX. *The Occurrence of Irregularities of Bleeding Related to the Duration of Therapy*

Cycle	Acoocen				Volidan				Wy 3707			
	No. of Cycles	BTB or Spotting		Cases of Amenorrhoea	No. of Cycles	BTB or Spotting		Cases of Amenorrhoea	No. of Cycles	BTB or Spotting		Cases of Amenorrhoea
		N of Cases	%			N of Case	%			No. of Cycles	%	
1	28	4	14	1	69	18	26	1	62	6	10	
2	25	1	4		64	11	17	1	58	4	7	
3	25	3	12	1	63	11	17	1	56	4	7	1
4	22	2	9		62	10	16	3	55	3	5	
5	22	1	5	1	59	7	12	3	55	2	4	1
6	21	2	10		58	9	16	2	52	2	4	
7	21	1	5		55	8	15	2	50	3	6	
8	21	2	10		54	4	7	5	47	3	6	
9	19	1	5		50	7	14	3	45	1	2	
10	7	1			43	6	14	2	39	1	3	
11	4				39	4	10	2	28	2	7	
12	2				25	4	16	1	10	1		
13					10	4			6			
14					3	1			4			
15									1			

Table X. *The Influence of the Medication on Pre-existing Dysmenorrhoea*

Change of Dysmenorrhoea	Number of Cycles on Each Drug in Per Cent		
	Aconcen	Volidan	Wy 3707
Disappeared	47.9	54	4 3
Decreased	41.9	44.4	43.9
Unchanged	5.4	0.9	12.9
Increased	4.8	0.7	.9

Table XI. *Changes in Libido and in Frequency and Enjoyment of Sexual Intercourse among 124 of the Participants*

	Number of Patients Who Observed		
	Increase	No Change	Decrease
Libido	9= 7 %	83=67 %	32=26 %
Enjoyment	76=6 %	40=32 %	8= 6 %
Frequency	3 =25 %	74=60 %	9= 5 %

rhoea. Table IX shows the frequency of these irregularities. The cases of BTB and spotting were grouped together.

In spite of the fact that the material is limited, it was quite evident that the irregularities were greater with Volidan than with Aconcen and Wy 3707.

Dysmenorrhoea

It is well known that dysmenorrhoea usually diminishes or disappears during oral contraceptive therapy. In this series, 118 women admitted to this symptom at their first visit. Table X shows the changes that occurred during treatment.

Effect on Sex Life

During the last visit before the trial ended, 124 of the participants were asked if they had observed any change in libido or in the enjoyment and frequency of sexual intercourse. Libido is here defined as the desire for sexual contact while enjoyment

Table IX. *The Occurrence of Irregularities of Bleeding Related to the Duration of Therapy*

Cycle	Acomcen				Volidan				Wy 3707			
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2	25	1	4		64	11	17	1	58	4	7	
3	25	3	12	1	63	11	17	1	56	4	7	1
4	22	2	9		62	10	16	3	55	3	5	
5	22	1	5	1	59	7	12	3	55	2	4	1
6	21	2	10		58	9	16	2	52	2	4	
7	21	1	5		55	8	15	2	50	3	6	
8	21	2	10		54	4	7	5	47	3	6	
9	19	1	5		50	7	14	3	45	1	2	
10	7	1			43	6	14	2	39	1	3	
1	4				39	4	10	2	28	2	7	
12	2				25	4	16	1	10	1		
3					10	4			6			
14					3	1			4			
15									1			

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Frequency	3 = 25 %	74 = 60 %	9 = 5 %

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Table XII. *Results of the Transaminase Determination*

	Results of the Treatment-determinations on Patients Using		
	Aconcen	Volidan	Wy 3707
SGOT			
Number of determinations	85	254	211
Number of elevated values	1=1.2 %	9=3 5 %	21=10.0 %
Maximum values in units	42	70	190
Mean of elevated values in units	42	54	68
Number of values higher than 100 U	0	0	1
SGPT			
Number of determinations	85	254	211
Number of elevated values	4=4 7 %	18=7 1 %	36=17 1 %
Maximum values in units	80	167	300
Mean of elevated values in units	59	75	100
Number of values higher than 100 U	0	4	11
Number of patients controlled	26	68	59
Number of patients with elevated SGOT values on at least one occasion	1=4 %	4=6 %	9=15 %
Number of patients with elevated SGPT-values on at least one occasion	4=15 %	8=12 %	16=27 %

of intercourse refers to the patients actual feelings during the act. There were small differences between the three groups and the answers were therefore aggregated in Table XI

All 19 women with decreased frequency of sexual intercourse also had decreased libido The participitants not included in Table XI were mostly those who left the trial prematurely

Serum Transaminases

With a few exceptions blood was drawn at each visit for determination of SGOT and SGPT The upper limit of normal was set at 40 units for both tests. All pretreatment determinations were within the normal range Table XII shows the results of the 550 transaminase determinations during the treatment

Fig 3 was obtained by plotting the frequency of abnormal SGPT-values against the five-day period in which the blood was

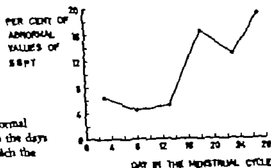


Fig. 3. Incidence of abnormal SGPT-values referred to the days during the cycles on which the tests were done.

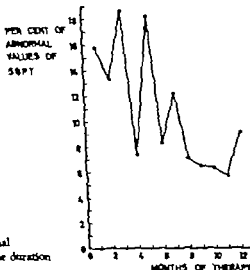


Fig. 4. Incidence of abnormal SGPT-values referred to the duration of treatment.

drawn. Fig. 4 shows the frequency of abnormal SGPT-values in relations to the duration of treatment. It may be seen that there was a tendency towards an increased incidence of abnormal values towards the latter part of each menstrual cycle and that the abnormalities were more frequent during the early months of therapy

Discussion

It is evident that the frequency of abnormal bleeding was greater with Volidan than with the other two drugs The gestagenic substances in Volidan and Aconcen, megastrol and chlormadinone respectively are similar but seem to have different progestogenic activity (Dominguez *et al*, 1962 Greenblatt *et al.*, 1963) which may explain the poorer cycle control with Volidan. This may however also be due to Volidan's lower oestrogen content

The amounts of both gestagen and oestrogen are small in Wy 3707 In spite of this the cycle control was good which suggests that the 19-norsteroid in this drug has a strong gestagenic action

Table VIII and Fig 2 convincingly demonstrate that users of oral contraceptives usually get lighter and shorter periods a valuable effect in patients with menorrhagia and iron deficiency anaemia

The effect of oral contraceptives upon mental functions is still under discussion. It is striking that in this material a significant proportion of the participitants developed mental irritability associated with depression. These changes, which were often a cause of domestic tension were frequently first noted by close relatives

Authors vary in their opinion about the effect of oral contraceptives on libido and related functions Zell *et al* (1964) found a definite improvement in sexual adjustment among 240 women using Enavid and Goldzieher (1962) observed increased libido in 47 per cent of his material but no cases of decrease Similiar experiences were reported by Tyler *et al* (1961) and by Rice-Wray *et al.* (1962)

However Hauser *et al* (1965) found decreased libido in 37 to 50 per cent and increased libido in 12 to 24 per cent of their cases. Kaiser (1963) observed a 10 per cent incidence of psychologic disturbances in users of Anovlar and Lyndiol including cases with diminished libido Pincus *et al* (1959) reported that one fifth of their cases had a decrease of libido and another fifth an increase Most authors however observed no significant changes (Brehm 1964 Mears, 1963 Mears

and Grant, 1962 Swartz et al 1963 Turpeinen, 1964) In this material one quarter of the women experienced a decrease in libido 6 women found their diminished libido so disturbing that they left the trial.

The observed adverse effect of oral contraceptives on libido may have several reasons. Progesterogens, unlike androgens, may have a directly depressant effect (Kupperman 1961) In addition, psychological factors probably operate It is believed by some that every woman consciously or subconsciously sees pregnancy as the ultimate aim of every intercourse To remove the possibility of this goal may well be the reason for a disturbance in libido.

In this series, about 2 patients out of 3 were of the opinion, that their enjoyment of intercourse increased during the therapy This favourable change is probably due to the great reliability of oral contraceptives, an effect which is achieved without any of the disturbing modifications in the sexual act required with most other methods of birth control.

Considerations such as these emphasize the importance of remembering that oral contraceptives not only have somatic actions but also may affect a woman's psychological adjustment to her marriage and the world beyond it.

It has long been known that 17-alkyl-substituted anabolic steroids may cause impaired liver function and even jaundice. Most oral contraceptives contain 17-alkyl-substituted steroids. The report of Eisalo et al (1964) about increased transaminase activity in postmenopausal women given oral contraceptives stimulated systematic investigations in this field. Many authors mostly from Finland or Sweden found abnormal liver function tests, especially elevated transaminase values, in oral contraceptive users (Palva and Mustala, 1964 Tyler 1964 Larsson-Cohn, 1965 Borglin 1965 Stoll et al 1965 Eisalo et al. 1965 Knutsson et al. 1965) Other investigators found no abnormalities (Linthorst 1964 Rice-Wray 1964 Swaab 1964 Swayer and Little, 1965) A growing number of jaundice have also been reported often in women who earlier had idiopathic jaundice or pruritus during pregnancy (Cullberg et al 1965 Larsson-Cohn and Stenram, 1965

Thulin and Nermark, 1966 Larsson-Cohn and Stenram, 1966 Lundbergh, 1966)

In this investigation, 18 6 per cent of the participitants had abnormal values of SGPT on at least one occasion. It was shown earlier (Larsson-Cohn 1966) that the frequency of abnormal values varied with different drugs. In spite of the fact that Wy 3707 contained the least amount of active substances the frequency and level of raised transaminases was higher than with the 2 other drugs. In an earlier investigation on 1288 blood samples from 699 women using different oral contraceptive agents for 3520 cycles (Larsson-Cohn 1966) it was shown that the frequency of abnormal SGPT values was lowest during the second week of the menstrual cycle that is about one week after the 7 days without tablets. It was suggested that the reason for this was a rapid regression of elevated values during this interim. This series is much smaller but a similiar trend was evident. The most striking difference, however is that there was a minimum incidence of raised values already during the first 5 days of the cycle

There was also a higher incidence of abnormal SGPT values near the onset of the therapy This tendency was not so pronounced in the earlier series The diminishing frequency of abnormal values could mean that after some time the liver adapts itself to the new steroids

SUMMARY

The results of a clinical trial are reported in which 165 women used three different oral contraceptive agents Aconcen Volidan and Wy 3707 during 1442 cycles No pregnancy occurred. A total of 16 per cent left the trial because of side reactions Volidan gave poor cycle control but tended to cause weight increase less than the other two drugs. A quarter of the women had the opinion that their libido diminished during the therapy but 76 per cent experienced increased enjoyment of sexual intercourse. In all 550 determinations of SGOT and SGPT were made. From 12 to 27 per cent of the women had abnormal

SGPT-values on at least one occasion. The frequency of elevated values was at a minimum during the first part of the menstrual cycle and there was a trend towards a diminishing frequency of elevated values with an increase in duration of treatment.

Acknowledgement

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VAGINAL METASTASIS OF HYPERNEPHROMA

Report of three cases

BY

T. Å. NERDRUM

The term hypernephroma was introduced by Grawitz who thought that this tumour had its origin in suprarenal remnants within the kidney substance. Although this theory was subsequently abandoned, the name given to this tumour has been retained and is in general use. However several synonyms are nowadays used in the literature, such as adenocarcinoma renis clear cell carcinoma of the kidney or "hypernephroid cancer of the kidney Böttiger (1962) suggested calling it simply renal cancer since no other carcinomatous growth would be likely to occur primarily in the renal parenchyma. For the sake of clarity only the term "hypernephroma will be used throughout this paper

Hypernephroma, earlier considered a urologico-surgical disease —which in the therapeutical sense it still is—has in recent years become a subject of increasing interest especially in the field of internal medicine owing to the recognition of several early symptoms of a non-urological nature. (Böttiger 1962.) Such symptoms were observed in abundance in one of the cases described below (Case No. 3)

The tumour under discussion should also be of interest to gynæcologists because more often than any other tumour occurring primarily outside the pelvic area, it also metastasizes in the vagina and adjacent parts of the vulva. Although these metastases are very infrequent, their significance in establishing

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BY

T. A. NEDRUM

The term hypernephroma was introduced by Grawitz who thought that this tumour had its origin in suprarenal remnants within the kidney substance. Although this theory was subsequently abandoned, the name given to this tumour has been retained and is in general use However several synonyms are nowadays used in the literature, such as adenocarcinoma renis clear cell carcinoma of the kidney or "hypernephroid cancer of the kidney Böttiger (1962) suggested calling it simply renal cancer since no other carcinomatous growth would be likely to occur primarily in the renal parenchyma. For the sake of clarity only the term "hypernephroma will be used throughout this paper

Hypernephroma earlier considered a urologico-surgical disease —which in the therapeutical sense it still is—has in recent years become a subject of increasing interest especially in the field of internal medicine owing to the recognition of several early symptoms of a non-urological nature. (Böttiger 1962.) Such symptoms were observed in abundance in one of the cases described below (Case No 3)

The tumour under discussion should also be of interest to gynecologists because more often than any other tumour occurring primarily outside the pelvic area, it also metastasizes in the vagina and adjacent parts of the vulva. Although these metastases are very infrequent, their significance in establishing

the primary disease is sometimes decisive so that a wider knowledge of their clinical picture is desirable

For this reason the author has thought it appropriate to report on three cases of vaginal metastases from hypernephromas The author was personally involved in the treatment of one of these (Case No. 3) and the others were found in the Case Records of the Department of Obstetrics and Gynæcology of the Helsinki University Central Hospital and the Pori General Hospital for the years 1950–63

Case No 1 Hosp Records No 5693/54 A 69-year-old widow who for about 4 months had experienced indefinite pain below the right costal arch and who finally had a sudden profuse vaginal hæmorrhage following physical exertion She was consequently admitted to hospital where a biopsy specimen was taken from a bleeding vaginal tumour which, in the histopathologic examination proved to be a *hypernephroma metastasis.*

On Nov 11 1954 the patient was transferred to the urological department of the Pori General Hospital for treatment It was found that she had a fist-sized, slightly tender firm tumour below the right costal arch. Near the vaginal introitus there was a purplish tumour about 2.5×3 5 cm and a smaller one a little deeper These vaginal lesions were excised and examined histologically Result: *Hypernephroma metastases.*

The patient had a very high ESR=138 mm/hour and clinically evident anæmia. Hb 54 per cent (Sahli) erythrocytes 2.81 mill./c.mm, leucocytes 11,850/c.mm Lues reactions: – non-protein nitrogen 30 mg per cent. Intravenous urography gave indications of a hypernephroma in the right kidney A right nephrectomy was performed and a tumour about 12 cm in diameter was found, it had developed from the caudal part of the kidney and adhered so firmly to its surroundings that complete excision was impossible *Histopathologic diagnosis of this tumour Hypernephroma.* After receiving 17 post-operative applications of roentgen therapy the patient was discharged. Unfortunately during the entire period in hospital no lung or other X-ray examinations were made with a view to revealing metastases elsewhere. The subsequent history of this case is regrettably unknown.

Case No 2 Hosp Records No 2105/I/57 A 67 year-old former offi c clerk who on Sept. 30 1957 was admitted into Dept of Obstetrics and Gynæcology Helsinki University Central Hospital because of *considerable bleeding from the genital tract* during July September She was also tired lacked appetite and had lost a great deal of weight On admission the patient s condition was poor she had a palpable hard nodular tumour sited to the right in the epigastrium and extending from the costal arch almost to the level of the umbilicus. Blood pressure 180/ 00 mm Hg.

Gynaecological examination. Projecting from the distal part of the anterior vaginal wall was a soft, purple tumour the size of a thumb-phalanx and having a stem about the thickness of a pencil. Abundant bleeding occurred during examination. Otherwise there was nothing of note regarding the genital organs except senile atrophy The vaginal tumour was extirpated, histological examination gave the result: *Metastatic carcinoma (hypernephroma) in the vagina.* On Oct. 1 957 an intravenous urography was performed but, owing to poor excretion of the contrast medium this was of no diagnostic value. As it was found that no further treatment of the primary tumour was possible, the patient was discharged on Oct. 21 1957 According to information received by the Clinic, the patient did not die until 196 the cause of death being *hypernephroma.*

Case No. 3 This patient, the wif of a farm manager was 60 years old, when, on March 28, 963, she was admitted for the first time to the Dept for Internal Diseases of the Pori General Hospital. She was subsequently readmitted on 7 different occasions to various departments of the hospital, (Hosp. Rec. Nos. 3 7/63, 4130/63, 4430/63 4583/63, 5785/63, 306/64 and 1421/64) Prior to the illness described below the patient had for six years been receiving reserpine treatment for high blood pressure which had dropped from 280 to 30 mmHg systolic pressure. An X-ray examination in 1960 showed that she had gallstones, which were not particularly troublesome: otherwise, her general health was satisfactory

In August 1962 the patient developed a severe cough and was treated by her local doctor who diagnosed pleurisy on the right side In November of the same year the patient again had an infection of the respiratory organs with an almost constant rise in temperature and cough. In January 963 the same doctor again diagnosed pleurisy on the right side. In February 963 the patient was treated at her local hospital for coliform infection of the urinary tract. At the same time she had an ESR of 30 mm/hour severe night sweating and persistent rise in body temperature. In an X-ray picture of the lungs there appeared to be a shadow at the base of the right lung. At the same time the patient had jaundice and her gall-bladder was tender to palpation Her evening axillary temperature still stood about 38 C. shortly before admission to the Pori General Hospital while her Hb was 70 per cent (Sahli)

Condition of the patient on admission to hospital 28 3 63

General condition good. BP 245/ 25 mmHg. Other findings normal including body temperature. *Laboratory tests:* ESR during the whole period in hospital was extremely high, varying between 126–143 mm/hour' Hb 105 4 per cent Blood picture otherwise normal except for thrombocytes at 5 ,500. Blood coagulation time 4 min bleeding time 5 min. 30 sec. *Thrombo-test 28 per cent Fibrinogen 1075 mg per cent* Serum iron 55 gamma per cent. Tests for liver and kidney function, normal. Urinary sediment, normal: culture non-haemolytic streptococci Paper electrophoretic

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been administered to the patient) leucocytes 2,700/cu.mm.

In September 1963 the patient was in hospital for postoperative radiotherapy receiving cytostatic treatment as well. Her general status was satisfactory but treatment had to be discontinued prematurely because of leukopenia.

The patient did not come for treatment again until Jan. 4 1964 when she had renewed vaginal haemorrhage. She had now developed a brittle tumour of the posterior vaginal wall from which there was profuse bleeding. This lesion was clearly a local recurrence of the metastasis excised previously X-ray pictures of the lungs indicated *numerous metastases* so that the planning of more extensive treatment had to be abandoned.

In Feb 964 the patient was admitted to hospital for the last time again because of haemorrhage brought about by the progressive dissemination of the vaginal lesions' the haemorrhage was arrested without much difficulty The patient *died at home on March 8 964.*

Main features of the cases. In all three cases an unexpected profuse vaginal hæmorrhage led to the discovery of the lesions. In two cases, the bleeding had been preceded by some kind of physical effort. In two cases hæmorrhage was also the symptom which brought the patient to the physician. In all three cases examination of the vaginal lesions led decisively to the diagnosis of the primary disease In at least one case at the time the presence of a vaginal lesion was confirmed, no recognizable tumour or metastasis was observed elsewhere In one case the primary tumour was sited on the left. Of the other two cases, in one instance it was definitely and in the other probably located on the right.

Discussion

The vagina is an organ in which extrapelvic tumours rarely metastasize even when there is general dissemination. Vaginal lesions of hypernephroma are also so rare that, in *general* they have not gained much clinical attention. For the same reason the reports published are mainly of isolated cases. Overbeck (1958) collected from the literature 44 cases and added one case of his own. He has also critically examined and discussed this material. Later Schofer (1961) and Fuchs (1963) each described one case

total protein 8.0 g per cent; albumin 40 5 per cent; *alpha$_1$-globulin 5.8 per cent alpha globulin 16 5 per cent* gamma-globulin zone broadened. *X-ray examinations.* Thorax normal cranium normal intravenous urography the possibility of a tumour in the left kidney could not be ruled out with certainty incidental finding cholelithiasis spinal column normal. Sternal puncture: normal. The patient was only treated for hypertension which went down to 185/110 mgHg There was no pyrexia during her stay in hospital. The patient was discharged at her own request on April 11 1963 Diagnoses were *Hypertension cholelithiasis.*

On June 15 1963 the patient was again admitted to the Department of Internal Diseases suspected of having a *hyperneph oma* While at home her subjective condition had been somewhat improved by the treatment for hypertension and anæmia but subsequent investigation showed that her ESR was still 1-8 mm/hour and that microscopical hæmaturia was present without bacteriuria. *General examination.* no loss of weight. Abdominal palpation. No abnormality B.P 190/115 mmHg Renal function tests normal Catheter specimen of urine: no bacterial growth Serum iron 48 gamma per cent. Hb 11 3 g per cent. *X ray examinations* both intravenous urography and retrograde pyelography were performed and also colography without any pathologic or even any suspect findings. On June 28th the patient had a *sudden and profuse vaginal hæmorrhage* when visiting the lavatory and subsequently had an immediate gynæcological examination performed by the author *Gynæcological status:* External genitalia. nothing of note. Just inside the introitus on the posterior vaginal wall there was a flat swelling 1 cm in diameter of a spongy consistency and covered with coagulated blood. Other parts of the vagina and other genitals were normal. As a result of the examination this swelling, the surface of which was ulcerated began to bleed *Rectal* examination showed the rectal wall to be intact The patient was now transferred to the *gynæcological department* and on June 29 1963 underwent complete excision of the bleeding lesion, under local anæsthesia. A specimen from the lesion was examined histopathologically Extract from the report: Judging from the histological structure the tumour *could* be a *hypernephroma metastasis.* Pathological anatomical diagnosis: *Metastasis carcinomatis in vagina* As the suspicion of a hypernephroma now seemed to be confirmed, the patient was transferred to the urologic department for treatment on July 7 1963. At this stage the patient's left kidney had become palpable Prior to this X rays had been taken of her lungs but no metastasis was revealed. ESR 129 mm/hour Hb 9 9 g per cent Body temperature normal as it had been all the time during her stay in hospital On July 12, 1963 a left nephrectomy was performed, and presented no difficulties. In the caudal part of the removed kidney a tumour the size of a woman's fist was found the histopathological diagnosis of which was *Hypernephroma* Postoperative progress was undisturbed Cytostatic therapy was begun on July 22 and the patient was discharged on the 25th The ESR was then 111 mm/hour Hb 12.2 g per cent, (stored whole blood had

between both kidneys seems to lend additional support to this theory However one cannot overlook the fact that in a considerable proportion of cases the hypernephroma is located in the right kidney (*cf* the cases reported by the author) and that the ovarian vein on this side cannot be the connecting link between the kidney and the vagina because here it empties into the inferior vena cava. Furthermore, in by no means every case has it been possible to demonstrate the presence of tumour tissue in the renal vein (*cf inter alia* Fuchs s case) following nephrectomy Neither have lesions always been shown in the region of the pelvic venous plexuses.

Moreover one would suppose that the introduction of tumour cells into the blood stream via the renal vein would rather lead to lesions in the lung or generalized metastasis. However this was not usually the case at the time the vaginal lesions were found.

The discrepancies in the foregoing theory have evidently struck earlier authors as well, for at least two other theories have been propounded in an endeavour to fill its gaps. One is based on the presumed retrograde transportation of tumour cells along periureteric lymph vessels, which would reach the vaginal area uninterrupted by the regional lymph nodes. The other theory is that the tumour cells reach the vicinity of the vagina through the urine. It appears, however that most authors have seriously doubted these hypotheses, and because of this it is not proposed to discuss them any further in this paper

Overbeck s material contains an interesting case which must be mentioned in this connection, for it was a case in which, notwithstanding eight years of thorough investigation of the patient, it had not been possible to demonstrate a primary tumour forming the source of the "hypernephroma metastasis found in the vagina. Although mention of the spontaneous regression of a hypernephroma does appear in the literature (Giertz and Larsson, 1963) it seems more likely that in this case it was a *primary vaginal tumour* the histological structure of which was difficult to distinguish from that of a hypernephroma. The type of tumour which could be considered in this connection, and which has indeed been encountered in the

Since in the majority of cases the vaginal lesions are confirmed before the primary tumour or other metastases have come to light this complication constitutes a valuable short cut to an early diagnosis in individual cases In the cases so far published the clinical course of this complication has been fairly consistent in its main features—profuse vaginal hæmorrhages often following some physical strain providing the main symptom The hypernephroma lesions have been characteristically sited in the distal third of the vagina and usually take the form of a purple sponge-soft, rather flat node—though in my case No 2 it was, exceptionally pedunculated In the cases presented here the main characteristics for the most part coincided with those described in earlier papers

In general when dealing with this subject the earlier authors held that hypernephroma lesions of the vagina are always metastases In this they are supported by the fact that almost without exception it had been possible in their cases to demonstrate a renal tumour In their discussions on the ætiology of the lesions the writers have merely sought to explain why and how hypernephromas metastasize in the vagina. In forming their theory they have paid especial attention to certain anatomical communications which exist or are thought to exist, between the kidneys and the vagina.

The theory which has received the most support is that based on the communication which exists through the left ovarian vein and plexus and the uterovaginal plexus between the left kidney and the paravaginal venous plexus It is a known fact that in the course of its development a hypernephroma often invades the renal vein, more or less filling this with the tumour mass. This "tumour thrombus can on the left extend beyond the ovarian vein which on this side empties into the renal vein. It is thus possible that cells detached from the tumour mass could find their way—assuming the presence of stasis in the pelvic venous system—with the retrograde blood flow as far as the paravaginal venous plexus there to settle and form metastases The fact that in cases with vaginal lesions of hypernephroma the primary tumour appears most frequently to be sited in the *left kidney* whereas it would otherwise be almost equally divided

The incidence of vaginal lesions in cases of hypernephroma is very low even though greater than that of other extra-pelvic tumours (Schäfer 1961) It may be mentioned for the sake of comparison that in 30–40 per cent of patients with hypernephromas, metastases were already present at the time of first admission for treatment (Böttiger 1962) According to information received from the Cancer Registry the incidence of hypernephromas cases in Scandinavia during 1958 was 544, which was as high as that of leukemia (Böttiger 1962) By setting against this the above-quoted percentage figure we obtain the incidence of metastasis of hypernephroma at first admission. This greatly exceeds the known number of cases with vaginal metastases. Age distribution in respect of vaginal lesions appears to favour the older age classes but in e.g. Schäfers case the patient was only 14 years old.

The prognosis in these cases is of course chiefly dependent upon the course of the kidney tumour This in turn is related to the tumour s degree of malignancy which is regarded as varying considerably according to its histological structure type (Giertz and Larsson, 1963)

Usually the disease has speedily proved fatal, especially after nephrectomy which to some extent seems to hasten the development of general metastasis. This circumstance is believed to be associated with manipulation of the renal vein during operation. Yet a surprisingly long survival may also occur (cf Case No 2) and, in exceptional cases adequate therapy has led to complete recovery In this respect, however the usual criteria may prove deceptive for metastases ha e been known to develop in a patient with a hypernephroma 20 years after treatment (Böttiger 1962)

Therapy implies whenever possible radical removal of the primary tumour by nephrectomy The vaginal lesions may either be removed surgically or destroyed by radiotherapy If extirpation is resorted to this must be done scrupulously for local recurrences are apt to occur The dissemination of tumour particles even during this operation is also a possibility which cannot be entirely excluded.

vaginal region too (Villa Santa 1964, von Numers *et al.* 1965) is a cancer deriving from residues of the mesonephric ducts, represented in the vaginal walls by Gartner's ducts. Tumours belonging to the same group have also been found in the ovaries (Teilum and Østergaard 1963), in the broad ligaments and the uterine cervix (Tóth, *et al.* 1964). Hypernephroma is also regarded as having a genetic association with the mesonephros (Boyd 1953), which explains the histological similarity in appearance between it and mesonephrogenic tumours. The most striking similarity lies in the presence of large pale cells called "clear cells" in the histological picture of all the tumours referred to.

Since apparently it can be difficult to distinguish histologically between a focus of hypernephroma and a primary mesonephrogenic vaginal tumour, one must also consider it possible that the latter could appear by chance in a patient with a hypernephroma and thus be diagnosed as a metastasis. The only conclusive proof of this would be whether residues from the Gartner's ducts could be demonstrated in immediate association with the vaginal lesion. In the reports so far published there is no mention of any such investigations having been made.

Neither can the possibility of a primary vaginal tumour be ruled out in cases where there is an apparently late, solitary hypernephroma metastasis. Such a case is the one reported by Fuchs (1963), in which the vaginal focus did not appear until a year after a quite small hypernephroma had been encountered as a chance finding in a kidney which was removed for other reasons.

It would appear that the possibility of primary hypernephroid lesions in the vagina could constitute an acceptable complement to the theories already expounded of the development of vaginal lesions of this kind. This would not necessarily exclude the vaginal tumour from being an aid to the diagnosis of hypernephroma but their coincidence would have to be explained.

It is surely clear that future pathologico-anatomical studies of these cases will have to be intensified and improved if we wish to establish the validity of any theory.

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THE EFFECT OF DRUGS CONTRACTING THE UTERUS ON THE FLOW OF URINE

A renographic study

BY

L. LAAKSO

Renal function tests, such as PAH (para-amino-hippuric acid) and Inulin clearance performed on a series of healthy human subjects showed a variation of 10 per cent in kidney function on successive days. This variation was regarded as physiological (Heidenreich, 1960). The results of such tests are influenced also by the patient's position, psyche, ingestion of fluids and various drugs (e.g. Winton 1952).

Corresponding variations have been elicited by renography calling for control of the relevant factors during this procedure (Isley *et al.* 1963). The patient's position and various tumours compressing the urinary tract obstruct the discharge of urine (zum Winkel *et al.* 1960). If discharging is inhibited by spasticity alone, spasmolytics can be used to correct the disorder (Staehle *et al.* 1963). During labour flow of urine into the bladder occurs between uterine contractions (Laakso 1964).

The effect of certain drugs influencing the renal tubules is evident in renogram. PAH has a depressing effect on the renogram of patients with kidney disease, but not in healthy subjects. This drug has been used as a diagnostic aid in ambiguous cases (zum Winkel *et al.* 1961). Noradrenaline lowers the amplitude, angiotensin slows down secretion and histamine accelerates it (Klapproth *et al.*, 1962). Chlorthiazide and its derivatives have not been found to affect the renogram (Gerble *et al.* 196). Chlorthalidone (Hygroton), in contrast, has an accelerating

SUMMARY

In this paper three new cases of vaginal metastases from hypernephromas are described. The most typical symptoms of this complication are stated as being sudden often severe hæmorrhage from a usually solitary purple soft focus in the distal third of the vaginal wall. Attention is drawn to the fact that this complication had in individual cases a notable diagnostic significance in that it often appears before the other symptoms of hypernephroma. Earlier hypotheses on the aetiology of vaginal lesions from hypernephromas are considered briefly. As a new hypothesis the suggestion is put forward that in some cases they may be primary and evolve from Gartner's ducts from which the so-named mesonephric carcinomas have also been found to develop these having a histological structure similar to hypernephroma tissue.

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effect on excretion (zum Winkel 1964) Sedatives influence the acceleration of excretion (Meade et al, 1961) Benemid which is excreted via the tubules has been found to have a mild decelerating effect on the rate of excretion (Wax et al 1962)

The object of the present study was to examine the effect on the function of renal tubules of drugs which stimulate contraction of the uterus In its structural composition synthetic oxytocin is very close to the antidiuretic hormone which causes re-absorption of fluid in the tubuli. Methylergometrinemaleate probably exercises no specific effect on the tubules.

Method and material

Renography was performed at the Central Hospital of Kuopio on parturients 2–9 days after delivery If the first renogram was normal the procedure was repeated the next day

All patients were supine during renography The kidneys were localised by urography and the position was checked immediately after the intravenous administration of the tracer Mobile detectors were directed to both kidneys and the third detector to the heart or the bladder

The measuring procedure was as follows

The detectors were 1 5 in × 1 in NaI(tl) crystals collimated in such a way that the measuring field at the kidneys was 12 cm in diameter and the crystal-kidney distance 25 cm. Adjustment of the rate meters range 3×10^4 counts per minute (cpm) range 10^4 corresponds to 10 μCi (with this range setting the dose used may be expected to give full scale deflection at maximum concentration) time constant 3 sec chart speed 3 mm/min.

The tracer used was Hippuran I 131 (ortho-iodo-hippurate) The quantity of tracer used was 30 μCi of Hippuran I 131 diluted with sterile isotonic saline solution to 0 5–1 0 ml (for pregnant women the quantity was 10 μCi) Accumulation in the thyroid of the patient of the free iodine (about 3 per cent) present in the Hippuran solution was inhibited by giving the patient 10–15 drops of Lugol's solution before the test

0 2 mg (1 cc) of methylergometrinemaleate (Methergin Sandoz) was administered intravenously to 12 patients during or after the

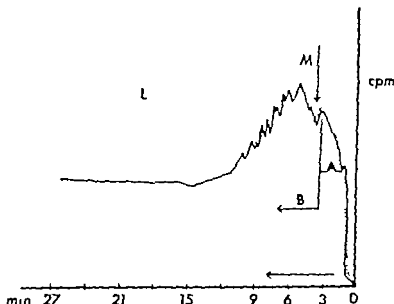
peak of the secretion. Similarly in 10 patients 2 IU of synthetic oxytocin (Syntocinon Sandoz) was administered during or after the peak of secretion. A further 5 patients were examined during labour (5 IU Syntocinon + 500 cc glucose – 20 drops/min)

Results

The second, secretory phase of the renogram (Fig 1 A) illustrates the functional ability of the tubules to take up the tracer Hippuran, from the blood. Neither Methergin nor Syntocinon had any effect on secretion, as compared with the control renogram. On the other hand, both drugs had an inhibitory effect on the emptying phase (Fig 1 B) whereas the tracer was excreted freely in the control renograms (Figs. 1–3) This effect is transient. Injection of Syntocinon does not prolong the half-time of excretion (normal time is 18 minutes according to zum Winkel 1964) During Syntocinon infusion have not been found emptying in the bladder according to the cystogram during the test. The injection of Methergin had a slight inhibitory effect in 9 cases on the excretory half time. In two cases there was a prolonged inhibitory effect on both kidneys and in one case on the right kidney only Although both Methergin and Syntocinon exert a similar effect on the emptying phase of the renogram, the mechanism of this effect is probably different in both groups

Discussion

The antidiuretic effect of Syntocinon has long been known (van Dyke et al 1957) This pharmacological effect is manifest with large doses It is understandable as the structural composition of Syntocinon and antidiuretic hormones are almost the same (van Dyke 1957) Syntocinon may even cause severe oliguria in man (Shipman et al 1964) Only in animal experiments has it been found to have a diuretic effect (Mertz, 1960) and to increase glomerular filtration but not diuresis (Horster 1960) The mechanism of this effect is still uncertain (Berde 1959) Von Keis r and zum Winkel (1962) have found in animal experiments that Adiuretin has an inhibitory effect on the emptying of the kidney pelvis and the proximal ureter Renography in



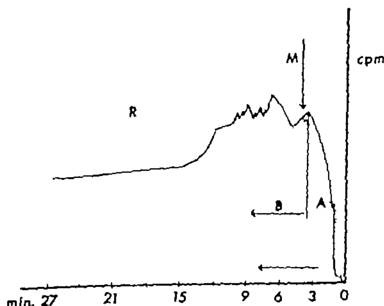
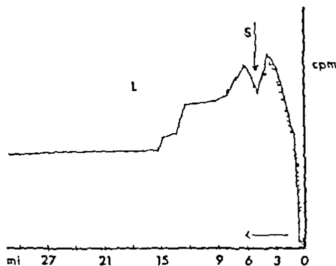


Fig. 1. Effect of Methergin on the renogram (3 days post partum)
Abbreviations: L = Left kidney. R = Right kidney. M = Methergin injection. S = Syntocinon injection, A = Secretion phase. B = Excretion phase. cpm = counts per minute. min. = minute (1 cm = 3 min.) —— = control renogram. — = renogram + Methergin or Syntocinon injection. × × × = control cystogram, – – = cystogram during Syntocinon infusion.



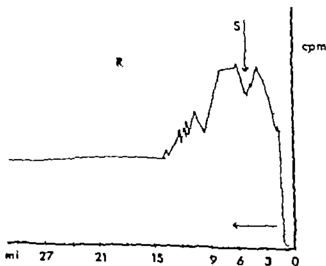
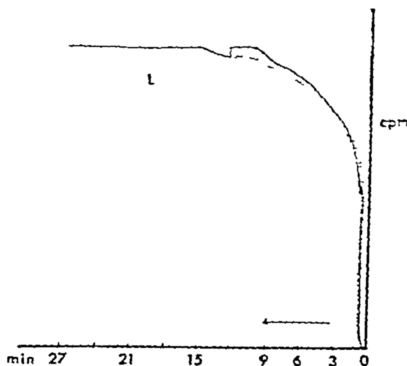


Fig. Effect of Syntocinon on the renogram (3 days post partum)



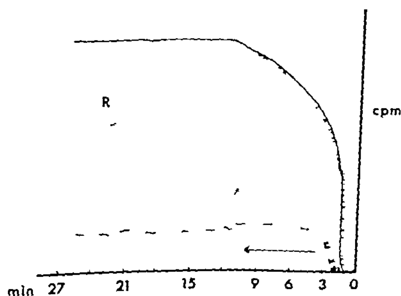


Fig. 3. Effect of Syntocinon infusion on the reno-systogram.
(Before delivery)

animals showed that Adiuretin (0.25 E pro kg) has an inhibitory effect on the excretory phase if the renogram was inhibited after injection of Syntocinon. As the secretory phase showed no change from the controls it is obvious that Syntocinon does not affect the ability of the tubules to concentrate the tracer nor does it affect the circulation. Syntocinon may however inhibit the tubular excretion of hippuran, increase the reabsorption of the tracer or cause contraction of the ureters.

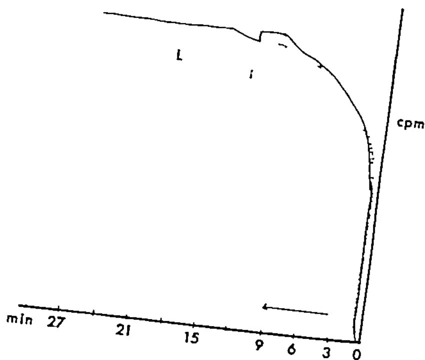
As judged by renography the inhibitory effect of Syntocinon on the flow of urine is most marked immediately after delivery. Seven μ-units per minute suffices to produce an antidiuretic effect in the parturition phase (Douglas, 1965) whereas the amount required to reproduce a corresponding effect in non-pregnant women is 200–300 μ-units per minute (Thomson 1960). In the doses used in our tests (Syntocinon 2 IU or Methergin 0.2 mg iv) no effect on the renogram was found in non-pregnant women. Douglas (1965) contends that Syntocinon infusion may aggravate the situation in pregnant women suffering from renal damage. From our results we agree with the opinion of Douglas.

Methylergometrine has a mainly contracting effect on the uterine muscle (Rauramo *et al.* 1954). This effect is strongest immediately *post partum*. Urography during pregnancy has shown that the ureters are dilated. Their muscle fibres have become hypertrophied during pregnancy as a result of hormonal influences (Kehrer 1952). Methergin may effect these cells more readily than usually. As the hypertrophy of the ureteral muscle gradually resolves, the effect of Methergin is weakened.

SUMMARY

Renographic studies showed that injection of synthetic oxytocin had a transient inhibitory effect on the flow of urine. The effect appeared to be most sensitive during Syntocinon-infusion before labour and immediately post partum. The injection of methylergometrinemaleate had a slight transient inhibitory effect on the excretory phase of the renogram immediately after delivery.

My thanks are due to Physicist A. Rekonen who has given valuable technical help.



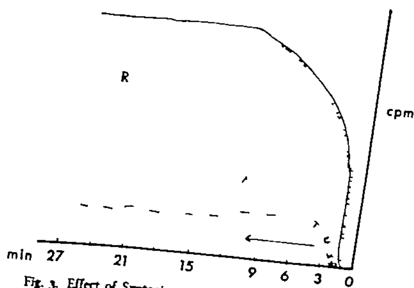


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